1986

Secondary sex ratio in Daphnia magna (Crustacea, Cladocera).

David Montgomery Barker
University of Windsor

CANADIAN THESES

THÈSES CANADIENNES

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

SECONDARY SEX RATIO IN *DAPHNIA MAGNA*

(CRUSTACEA,CLADOCERA)

by

© David Montgomery Barker

A Thesis
submitted to the Faculty of Graduate Studies
Through the Department of Biology in
Partial Fulfillment of the requirements
for the Degree of Master of Science
at the University of Windsor.

Windsor, Ontario, Canada

1986

ISBN 0-315-29280-6

GRADUATE COMMITTEE

Approved by:

(Dr. P.D.N. Hebert, Chairman)

(Dr. R.T. M'Closkey)

(Dr. M. Starr)

...I knew
a million ways the jungle might have been
meaner and smarter.

--Leonard Cohen, Order.

ABSTRACT

SECONDARY SEX RATIO IN DAPHNIA MAGNA

(CRUSTACEA,CLADOCERA)

by

David Montgomery Barker

The fresh-water crustacean order Cladocera exhibits two traits which make it of interest for study of adaptive secondary sex ratios. These traits are the parthenogenetic production of both males and females, and the influence of environmental conditions on the occurrence of males.

This study examines natural secondary sex ratios (SSR) (ratio of neonate sexes upon their release from the brood chamber) in four populations of Daphnia magna. The populations inhabited rock pools in the vicinity of Churchill, Manitoba. During the summer of 1984 parthenogenetic females were collected each day from the populations. A daily record was kept of brood size and the proportion of male neonates that these females released. A group of females was kept after the release of their first brood in order to determine the sex of their subsequent brood. A survey of D. magna populations provided estimates of juvenile sex ratios in 21 ponds. In 1985 three experiments were performed with the aim of determining specific factors responsible for brood sex, which would also determine an individuals' SSR's.

The study revealed that the populations switched from producing only female offspring to producing roughly 50% male offspring. Broods were predominantly unisexual. Estimates of juvenile sex ratios from the population survey corroborated the estimates of neonate sex ratios. The observations on successive broods show considerable flexibility in an individual's brood sexes. The three experiments testing factors affecting brood sex appear to discount an hypothesized internal cycle of sex-determining physiological states in the female, egg responses to temporally cycling environmental conditions, and egg responses to population density experienced by the mother.

The study concludes that, based on literature reports, the observed sex-allocation pattern may be general for cladocerans. Cladocerans probably practise facultative sex-ratio manipulation with sex being determined genetically, or through interactions between maternal physiology and the egg's genome. Future studies should aim to discriminate between the last two possibilities, and to measure the sex-ratio variation among individual mothers.

ACKNOWLEDGEMENTS

My first acknowledgement of gratitude is an unconventional one to the animals I was introduced to on my arrival in Windsor, and with whom I have since tried to form a sympathetic, though regrettably parasitic, relationship. Not secondly, but alongside this acknowledgement, I am indebted to my supervisor, Dr. Paul Hebert, who introduced me to the Cladocera, and who has molded my perception to better understand biological theory, and cladoceran biology. I am also happy to thank him for his unique friendship during my time here, and in the future.

I am sincerely grateful to my other committee members who are Dr. Robert M'Closkey, and Dr. Meyer Starr. They have both been interested, and helpful with my work.

Very little of the data in this thesis would be present if it were not for the careful and tireless efforts of Barbara Burleigh during the 1984 summer field season in Churchill. The majority of the pond survey data was collected by An van de Vel on her visit to Churchill, and I am indebted to her for what has become a very useful body of data.

Certain friends I have made while in Windsor have each contributed to what I have learned about biology, though in diverse ways. They are Barbara Burleigh, Luise Hermanutz,

Guy de Lanversin, Marlene Schwartz, Steven Schwartz, and Jess Zimmerman. These people, and a few others, have also, each in their own way, taught me much about humanity. These were certainly the most important lessons I learned.

I thank the Woods and Figurski families for many happy moments in Churchill, the staff at the NRC Rocket Range for their tolerance and company in their work place, and the DINA Northern Studies Training Program and Northern Studies Group at the university for the financial support for my field work. The university Computer Centre staff were very helpful with several inter-facing problems between this user and the IBM system.

PREFACE

The term 'sex ratio', and phrases including it, have been used a number of different ways in the literature. To avoid confusion, definitions for these terms as used in this text follow here. Sex ratio will mean the proportion of male individuals in a population, or a sample. Sex ratio will always be expressed as a decimal fraction, the result of dividing the number of male individuals by the total number of individuals (i.e. male + female). Primary sex ratio will mean the proportion of male conceptions, or male eggs released from the ovary (since in parthenogenesis conception does not take place). Secondary sex ratio (SSR) shall describe the proportion of male offspring at the end of parental expenditure in those offspring. The parental sex-allocation ratio (PSAR) will mean the proportion of investment by parents in male offspring as measured at the end of parental care (see the Introduction). The term SSR is used as a general term; sometimes when PSAR is a more accurate description. It is generally assumed, for simplicity, that the sexes have equal costs of rearing. Tertiary sex ratio describes the proportion of reproductively mature males that are in the population of reproductively mature individuals. It is also used in a general sense to describe sex ratios among the population members which are no longer being cared for by parents.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

The introduction is divided into four sections. These review information necessary for the present work and describe the rationale and context of the study and the experimental designs. Section one is an introduction to the conclusions of the theories of sex-ratio evolution. Three topics are covered; 'Fisherian' sex-ratio theory, facultative sex-ratio manipulation, and sex ratio under environmental sex determining systems. The following section reviews the life cycle and biology of cladocerans with specific details pertaining to Daphnia magna. The treatment is not a complete one, but covers the known facts of cladoceran biology which are relevant to the problem of their sex-ratio evolution. The third section presents the debate and evidence on the mode of sex determination in cladocerans and Daphnia in particular. The final section outlines the experimental rationale, and justifies the interest in cladoceran sex-ratio evolution.

"Fisherian" Sex-Ratio Evolution

Fisher (1930, pp 158-60) first postulated a selective mechanism for the maintenance of population and individual sex ratios of 0.5. Parents inevitably invest a certain amount of their limited resources in offspring to get them started in life. Such investment includes, at least, the provisioning of an egg and the production of sperm, but may

also be supplemented by expenditures, on the part of one or both parents, of time and energy in rearing. At some point this investment must cease. Fisher considered the reproductive value (potential for producing descendents) of a generation of offspring at the point of cessation of parental care. Because each sex contributes one half of the gene pool of the next generation (every offspring has only two parents, one male, one female), the reproductive value of all the males in a generation must equal that of the females. The parents of a generation should therefore not invest, on average, more than half their resources in one sex. If one sex costs less to produce, then individuals which have a tendency to produce that sex in excess are purchasing a greater proportion of the reproductive value of the next generation for a smaller expenditure. If the tendency to bias offspring sexes is heritable, such a trait will increase its representation in subsequent generations. If the sexes suffer differential mortality before the end of parental care, selection will favour parents with a primary sex ratio that is biased in favour of the sex suffering the higher mortality, because, on a per offspring basis, it is the cheapest to produce. The equilibrium secondary sex ratio will be 0.5, as will be the parental sex-allocation ratio (PSAR). On the other hand, if the sexes require differential investment for

growth (i.e. not due to mortality) then, at equilibrium, the cheaper sex is overproduced at conception, and may be numerically dominant at the end of parental care. Although the SSR is biased in favour of the cheaper sex, the PSAR is still 0.5. Any differential mortality in the sexes after the end of parental care cannot affect the pattern of parental investment, although it may force adaptive changes in other life history characters, or the mating system.

Fisher's argument rested upon these three fundamental assumptions:

1: There is a genetic component to variation in family sex ratios that is transmitted autosomally.

2: The population consists of individuals which all have an equal opportunity to mate with each member of the opposite sex (i.e. the population is randomly mating).

3: The male-female-parent (or rearing individuals) genetic relationships are symmetrical.

In a 1953 paper Shaw and Mohler framed Fisher's argument in genetic terms, and provided an algebraic model to describe selective pressure on sex-ratio traits carried by individuals in a population with a given sex ratio. They made the additional assumption that there was no parental investment after the egg was provisioned. The equation that Shaw and Mohler developed has the following form:

$$C = 1/4\ (m/M + f/F),$$

where C is the (genetic) contribution of a male parent to the generation of his grandchildren (his fitness), m and f are the numbers of male and female zygotes in his progeny, and M and F are corresponding values for the population of progeny. By setting the population sex ratio (M and F) at various values it is possible to find the progeny sex ratio which maximizes his fitness in that context. Selection equilibrates at a population primary sex ratio of 0.5.

Shaw and Mohler elucidated several important features in sex ratio evolution:

1: That whenever the primary sex ratio of the population is not 0.5 evolution brings it toward that point. As long as the population primary sex ratio is 0.5 there is no selection for sex-ratio genes regardless of their effects.

2: At equilibrium, a mutant gene which biases a family sex ratio will slightly bias the population sex ratio, and will therefore be selected against. The selection against such a gene may not be very strong, or will be non-existent if its effect is countered by other loci having the exact opposite effect through other individuals of the population. For this reason they predicted considerable variation for sex ratio among individuals in natural populations.

3: These principles hold if there is no parental care

(their simplifying assumption), if the sex-ratio genes are not sex-linked, and if there is no intergroup selection.

Shaw and Mohler's disregard of parental care in their model was considered unduly simplifying by Bodmer and Edwards (1960). They, therefore, developed an analytic model of Fisher's original argument that included the effect of parental care on sex-ratio evolution, and developed an expression for the selective advantage of an individual producing a given SSR in the context of the population SSR. They also examined the relationship between the genetic variance for sex ratio in a population and the rate of approach to the equilibrium, 0.5.

Kolman (1960) again restated Fisher's argument, and argued from his model that selection will not differentiate between a population of individuals each producing equal numbers of males and females, and a population in which half of the individuals are producing one sex and half the other. This agreed with Shaw and Mohler's (1953) conclusion that selection could allow considerable variation in individual SSR's provided that the population SSR was 0.5. Kolman (1960) concluded that only the mean SSR (population mean) is fixed and that a population may exhibit any degree of heterogeneity in family sex ratios. This property of sex-ratio evolution has figured importantly in studies on the variation in sex ratio

expected and found in natural populations, and in the theory of facultative sex-ratio manipulation.

The prediction of high variability in familial SSR's has not gone unchallenged. Verner (1965) pointed out that variance in sex ratio of a population can be maintained only if the system is not perturbed once reaching the population SSR of 0.5, a stable equilibrium point. Or, if the variance is increased it must be done exactly symmetrically. The population mean sex ratio must remain at the equilibrium value. The instability of this situation leads to a reduction in SSR variance at a rate inversely proportional to population size, and directly proportional to the magnitude of the variance (Bodmer and Edward's (1960) conclusions). Verner used Kolman's model to examine the stability of sex-ratio gene frequencies when each family SSR is 0.5.

In a 1967 paper Hamilton examined the assumptions of sex-ratio theory, and showed the effect of relaxing each on the evolution of sex ratio. I will only consider his results for situations in which the assumption of random mating fails. Hamilton proposed the following model population. Dispersing females settle on hosts, and deposit eggs on them. Several females may deposit their progenies on a single host. The eggs hatch, and the population of individuals on the host mate among themselves

before the inseminated females disperse to lay their eggs. The model assumes that fitness is proportional to the number of inseminations by sons plus the number of inseminated daughters. Hamilton asked the question; what is the sex ratio that an individual mother should produce to maximize her fitness. He was able to derive the following equation,

$$M = (\acute{n} - 1)/2n,$$

where M is the proportion of male offspring a mother produces to maximize her fitness, and n is the number of mothers using that host. When n is large (that is, the population approaches random mating) M approaches 1/2. In the extreme, when n = 1, then M = 0. Hamilton interpreted this to mean that the single mother's fitness depends entirely on the number of inseminated daughters dispersing, and that the mother should only produce enough sons as are necessary to inseminate their sisters.

Facultative Sex-Ratio Manipulation

Increased variance in sex ratio might be found in populations under selection for facultative sex-ratio manipulation (FSRM). FSRM might be found in situations where it is to the mother's (or rearing individuals') advantage, at certain times or under certain conditions, to modify the familial SSR away from 0.5. Such biases may

affect the lifetime SSR of an individual, or may only affect the expected binomial probability of an individual's offspring's sexes at one point in the reproductive period, a reverse bias occurring at another point in time. Burley (1982) has outlined the three types of situation under which facultative sex-ratio manipulation might be expected to be found. The first of these occurs if there are frequent shifts in population sex ratios away from 0.5. Her example suggests that the driving force in this situation is selection for an appropriate facultative sex-ratio response by a mother brought about through varying differential mortality in the sexes before the end of parental care.

The second situation favouring the evolution of FSRM occurs when the selected population SSR value varies temporally or spatially. This would occur, for example, in a population fitting Hamilton's (1967) model in which the number of females ovipositing on a host (n) varies.

The third situation favouring the evolution of FSRM covers a general class of situations which I will examine in some detail because of their bearing on the evolution of environmental sex determining systems. The idea originated with Trivers and Willard (1973) who proposed a model of sex-ratio evolution with three underlying assumptions:
1: The condition of young at the end of parental care tends

to be correlated with the mother's condition during parental care.

2: Differences in the condition of young at the end of parental care persist into adulthood.

3: The ratio of male and female fitnesses changes with female condition.

The latter assumption is important and is illustrated graphically in Figure 1. Trivers and Willard suggested that males might benefit more than females from a higher early investment in species in which males compete as adults for mates, and therefore have more to gain from being large. As females deviate from the mean adult female condition they should tend to bias their own sex ratio in order to produce more of the sex which benefits to the greatest degree from their present condition. One should find females in excellent condition tending to produce male offspring because those males will have an above average fitness as adults. If the mother produced females while in that condition her expected fitness would not be as high. These deviations should negate one another, resulting in a population sex ratio of 0.5 (Trivers and Willard 1973).

Evolution of Environmental Sex Determination

Charnov and Bull (1977) and Bull (1982) discuss the conditions under which environmental sex determination (ESD) is likely to evolve and the resulting evolution of

Figure 1. Hypothesized relationship between the quality of an offspring's early environment (horizontal axis), and its expected fitness (vertical axis) for the two sexes, which could lead to the evolution of facultative sex-ratio manipulation, or environmental sex determination. The figure is adapted from Bull (1983).

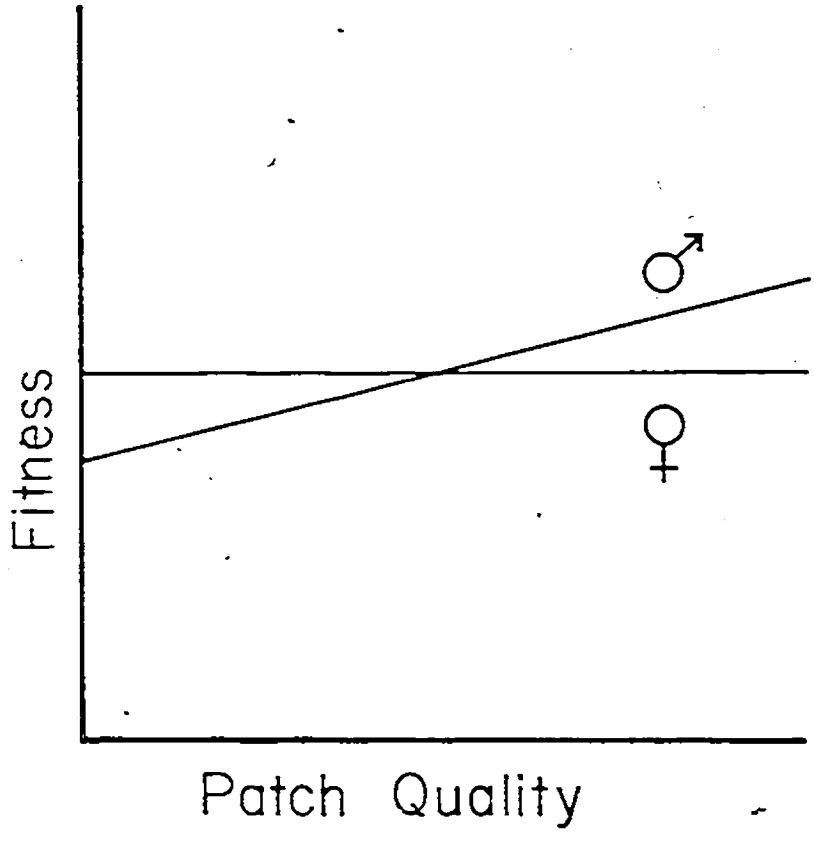


FIGURE 1

sex ratio. Their model depends upon two conditions. First, that an individual enters a patch early in life, the quality of which has a lasting effect on its fitness, and that the ratio of male/female fitness over all patch types varies. Mating occurs randomly outside of the patches. This condition is a restatement of the assumptions underlying Trivers and Willard's model. Secondly, the parents and offspring have no control over the patch in which the offspring finds itself. Thus, if sex were determined genotypically, some offspring might find themselves in a certain patch as the sex of lower fitness for that patch's quality. In such a situation ESD will evolve from a genetic sex determining system if there is some effect of environment on sex determination. Genes allowing males to develop in their appropriate (high fitness) patch type are selected for, and vice versa. Genes determining sex independently of patch type are eliminated from the population. The form of the evolutionary equilibrium is such that all individuals in patches below a threshold quality develop as male, all those above develop as female. This threshold is the intersection point of the two lines in Figure 1. Moreover, the equilibrium population sex ratio is not necessarily 0.5. This latter conclusion has not been adequately modelled.

Cyclic Parthenogenesis and the Life Cycle of Daphnia

Cyclic parthenogenesis (or heterogony) is a life cycle in which reproduction is accomplished at different times by sexual and asexual means. Females are capable of producing two types of egg, an egg which develops parthenogenetically, and an egg which requires fertilization for development. Asexual offspring may be produced by arrhentoky (haplodiploidy) in which males are haploid and females are diploid, or by diploid parthenogenesis in which both the male and female are diploid. Heterogonic populations exhibit from one to many asexual generations terminated by one sexual generation usually in an annual or seasonal cycle. Typically, parthenogenetic eggs are subitaneous (developing immediately), and fertilized sexual eggs, or early embryos arising from them, are a diapause stage in the life cycle. Heterogony is best exemplified in three invertebrate taxa; the rotifer class Monogononta, the insect superfamily Aphidoidae, and the crustacean order Cladocera. Heterogony is also considered descriptive of the life cycles of some trematodes, the Cynipidae (Hymenoptera), Micromalthus (Coleoptera), and the Cecidomyidae (Diptera), (White 1973, Bell 1982).

The general form of the Daphnia life cycle begins with the hatch of the over-wintering egg near the time of spring

thaw, or after a refilling of a pond. It appears that the diapause eggs invariably hatch as female individuals. A female becomes mature in the 4th or 5th instar, and proceeds to give rise to a variable number of parthenogenetic female generations. Each female, once mature, passes through a repeating cycle of brood release, moult, egg release (from the ovaries to the brood chamber), and incubation until the next brood release. During the incubation period the eggs develop into small versions of the adult, and are free swimming on their release, or become so in a matter of minutes (Scourfield 1943). The duration of a brood (or moult) interval is about 72h at 20° C and is strongly temperature dependent (see Hebert 1978). The interval between brood release, and release of a subsequent batch of eggs is short, lasting at most a few hours. Males and females pass through several pre-adult instars (4-5 in D. magna) before reaching reproductive maturity. At some point in the season male production begins, then females begin producing haploid sexual eggs, which must be fertilized by male sperm to develop. During copulation these eggs are released into a structure called the ephippium, a modification of the carapace surrounding the brood chamber, which is cast off during moult with the eggs contained within. The eggs undergo several cleavages (Ojima 1958), enter diapause, and the following spring

hatch into female offspring.

Environmental Sex Determination in Daphnia

There has been some discussion in the literature of the true nature of sex determination in the Cladocera. The most detailed studies of sex determination in the Cladocera are those of Banta and Brown (summarized in Banta et al. 1939) with Moina macrocopa. It is probably fair to extend most of their results to Daphnia species. Conditions of high density, low food levels, low temperature, or the presence certain chemical compounds were found to induce the production of male offspring. All of these factors appeared to have the common effect of lowering the mother's metabolic rate (Banta et al. 1939). The critical period for the effect of male inducing factors on parthenogenetic eggs was localized to a period some four hours (at 20°C) before release of the egg to the brood chamber (Banta and Brown 1929, but see also Banta and Stuart 1932). Groups of mothers treated with male inductive conditions after this critical period do not respond by producing increased numbers of males. This critical period occurs near to the time of the maturation division of the parthenogenetic egg, which begins about 1/2h before the egg's release, and is completed in the brood chamber (Allan 1928, Allan and Banta 1929). The timing of the maturation division in the development of the egg is the same for Daphnia pulex (Ojima

1958), and D. magna (Lumer 1937). During the maturation division a polar body is produced, which is either extruded from the egg, or disintegrates within it (Ojima 1958, Lumer 1937). Banta and Brown (1929), Banta (1931), and Banta et al. (1939) have discussed the possibility of an environmentally controlled segregation bias producing a (genetic) difference between the male and female genomes (see the Discussion below for such a system as it exists in aphids). The chromosomes of Daphnia and Moina are difficult to visualize, but to date there has been no identification of chromosome differences between the sexes. Allan (1929) showed that the male genome is of the same ploidy level as that of the female. The male sex is, therefore, not determined as a result of haploidy. Bacci et al. (1961) argued that synaptic behaviour observed during the maturation division of parthenogenetic eggs of Daphnia pulex provided the opportunity for induced segregation biases leading to male/female genetic differences. Hebert and Ward (1972) studied the electromorphs of three polymorphic enzyme loci in Daphnia magna, and found no evidence of crossing over during parthenogenesis, or of differences in male and female offspring of heterozygous mothers. Ruvinsky et al. (1978) detected differences in chromatin structure between males and females of D. pulex. They suggested that sex determination was dependent not on

the presence or absence of genetic factors, but on activity of male and female programmes within the genome (environmental sex determination). Most authors believe that cladocerans display environmental sex determination and that there is no genetic difference between males and females (Frey 1982).

Study Rationale

Heterogony provides an interesting system for investigation of sex-ratio evolution, and control mechanisms, because under parthenogenesis males are not needed, and are normally absent from the population. The onset of sexuality requires the expression of sex-ratio controls that had been suppressed, or inactive. The Cladocera are of particular interest because they are reputed to exhibit environmental sex determination, a trait which introduces interesting problems for the evolution of sex ratio, and a sex-ratio control mechanism (Bulmer and Bull 1982). To date all attempts to assess secondary sex ratios in natural populations of heterogonic species, or to study the manner by which sex-ratio control has evolved have been carried out on aphids (summarized in the Discussion). The present study forms an analysis of these problems as they relate to the Cladocera, based upon data collected from natural populations of Daphnia magna.

Experimental Design

The initial purpose of the 1984 field season was to attempt to verify a theoretical model of sex allocation by gathering data on the production of male offspring over the season. The model suggested that there would be an optimal time for parthenogenetic females to begin producing male broods in order for a clonal and population sex ratio of 0.5 to be produced by the time a switch to ephippial production was necessary. It was felt (a general impression gleaned from the literature) that all females behaved in the same way, and that once a female began producing male offspring she would not again produce female offspring. Thus, a population would be found to produce only male offspring at the end of the season. This latter prediction was not borne out by the observations on population sex ratios. These observations of the temporal pattern of secondary sex ratio are the first data presented in the results. It was apparent that the populations under study were not tending to 100% male production, but switched from a brief period of female only production into a period in which both males and females were produced. This suggested a response to a cue inducing sex-ratio (i.e. an adaptive ratio of male and female offspring), rather than a cue inducing males per se. The behaviour of individual females to this cue naturally became

interesting.

An attempt to follow the sex-allocation pattern of individual females for two broods was made in 1984. In 1985 three experiments were performed in an attempt to test three hypotheses of sex-ratio control advanced in Barker and Hebert (1986). The hypotheses rested on the assumption that individual females began to alternate the sexes of their broods in response to a sex-ratio cue. This assumption was itself an untested hypothesis, but such behaviour would have produced the population SSR patterns observed in 1984 (see Barker and Hebert 1986).

Experiment one was designed to test for an endogenous sex-alternation cycle, which was turned on by the environment, and produced alternating internal sex-determining environments. Experiment two was designed to test for actual sex-determining environments, which cycled over time producing alternating periods during which males and females would be determined before release to the brood chamber. Experiment three tested the hypothesis that the sex-determining environments were separated spatially in the environment, and that mothers would control their presence in each environment type thus controlling the sex of their offspring. The specific environmental parameter that was chosen was daphniid density, since animals are normally found to be clumped in distribution in a pond.

METHODS

Study Site

The study was conducted at a low arctic site near Churchill, Manitoba (58°47'N, 94°11'W) during the summer of 1984 (June 24 to September 10), and during August and September of 1985. The four populations of Daphnia magna studied in detail in 1984 inhabited neighbouring, but unconnected, rain-fed rock pools on a quartzite outcrop (Bluff A) on the Hudson Bay coast (see Good 1981 for a general description of the habitat). These rock pools support a diverse community of freshwater invertebrates, but the community of zooplankton in the four ponds was dominated by D. magna. The four ponds ranged in maximum volume from about 200 to 1100 litres, though volumes fluctuated greatly during the summer. Maps of the four study ponds appear in Appendix I. Seventeen other populations of D. magna were surveyed for population structure during August and September of 1984. These populations inhabited ponds located elsewhere on Bluff A, and on the Churchill Bluff (located on the coast, in the town of Churchill).

Population SSR's (1984)

Data collection began when the first male neonates were noticed in pond samples. Ponds were then sampled

daily using a small dip net. The dip net was drawn through the pond in an attempt to gather a large sample (upwards of 1000 individuals) from several locations in the pond. Between 15 and 30 females carrying parthenogenetic embryos in the brood chamber (brood sex determined in the pond environment) were selected at random from each pond sample, and isolated in 100-ml plastic cups containing filtered pond water. The subsampling from the pond sample was not rigorously randomized. Females were consciously chosen only on the criterion of carrying a brood, without discrimination as to the number of embryos they carried, or their own body size. The pond water used for culture was drawn from two nearby "tundra" ponds, and filtered through 64-micrometer Nitex mesh. The cups with mothers were kept outside, and experienced natural photoperiods, and temperatures until brood release. Each brood was counted, and each neonate sexed. On release from the brood chamber male neonates of D. magna are readily distinguished from females by the presence of enlarged antennules (Scourfield 1943). No significant neonate mortality occurred during the 18-to-30h period between neonate release, and examination. Mothers isolated on any one day carried embryos at various stages of development. However, all broods released in a single 18-to-30h period had their sexes determined during a similar 18-to-30h period some

days previously. The exact duration of the incubation time depended upon water temperatures, both in the pond, and in the cup. Sex ratios were calculated for day of release, and hence day of brood sex determination, rather than collection day. Mothers and young were ordinarily returned to their ponds after the observations were made.

The significance of a sample estimate of a population proportion may be determined by the methods of Croxton (1959). Detailed tables for 95% and 99% confidence intervals for sample estimates of proportions are available in Mainland et al. (1956). The confidence intervals provide a range of proportion values which one can be 95% (or 99%) confident include the true population proportion. The confidence intervals assume the sample was drawn randomly from the population, and depend only on the total sample size (n). All sex ratios calculated from the data of this study are provided with 95% confidence intervals drawn from tables, or calculated by Croxton's (1959) exact method.

Population Survey (Juvenile Sex Ratio)

During the months of August and September of 1984 samples were drawn from the four study ponds, and a larger group of ponds supporting populations of D. magna. These were large qualitative samples designed to estimate the proportions of the reproductive types of individuals in

each D. magna population. A dip net was drawn through the pond with care to sample from many areas within the pond. In most cases a second replicate sample was taken immediately after the first. Usually the whole sample was counted, however, a preset number of subsamples (5 to 10) were drawn from the sample in the process of counting. If these subsamples were homogeneous (i.e. chi-square test, $p <= 0.05$) the rest of the sample was not counted. The following types of individuals were identified and counted:

Ephippial Female	Female carrying an ephippium with eggs inside (ephippium is darkly pigmented).
Parthenogenetic Female	Female carrying parthenogenetic embryos in the brood chamber.
Non-reproductive Female	Females of reproductive age without embryos in the brood chamber, and without eggs in their ovaries.
Imminently Parthenogenetic Female	Female without embryos in the brood chamber, but with parthenogenetic eggs in the ovary. Carapace not modified in preparation to form an ephippium.
Imminently Ephippial Female	Female without embryos in the brood chamber but with ephippial eggs in the ovary. Carapace showing modifications in preparation for the formation of an ephippium.
Juvenile Female	Female without embryos in the brood chamber, without eggs in her ovaries and smaller

	than the smallest reproductive female.
Male	Presence of male secondary sex characters. Pubescence present on ventral margin of carapace, ventral margin of carapace well flattened (see Scourfield 1943).
Juvenile Male	Presence of male secondary sex characters. No pubescence on ventral margin of carapace, ventral margin of carapace not fully flattened (see Scourfield 1943).

Brood Series

A number of mothers from the population sex-ratio experiment that carried a second parthenogenetic brood, deposited after the release of the first brood, were re-isolated in 5-cm Petri dishes. Sex determination of the second brood occurred while the mothers were in the 100-ml cup, and thus were subject to natural photoperiod and temperatures, but unnatural density and food levels. The offspring of these broods were also enumerated, and sexed upon release.

Sex-Ratio Control:Endogenous Cycle Experiment

This experiment ran from August 15 to September 11 in 1985. A group of parthenogenetic females were drawn from pond 27 on Bluff A. The experiment began with 155 individual mothers. The mothers were isolated on (August

15) in 100-ml plastic cups containing filtered pond water (as in 1984), and were again kept outside. These females were ex-ephippial and first parthenogenetic generation individuals. They came from a second population for that season, which arose from eggs hatching after the pond refilled with water on June 22 (Neil Billington, pers. comm.). The pond re-dried on August 17 after the females had been removed. When a brood was released it was enumerated, and sexed, and the mother's culture medium was replaced with fresh pond water. After release of the first brood each female was given an identifying number so that her individual brood sequence would be known.

Sex-Ratio Control: Temporal Cycle Experiment

This experiment ran from August 19 to August 27 in 1985. The experiment was performed in pond A31 with the aid of the enclosure diagrammed in Appendix III. The enclosure provides a 'natural' pond environment for individual Daphnia by allowing the free flow of water, suspended particles, and dissolved compounds. The enclosure held isolated females for the purpose of identifying, with relative precision, when the sexes of their broods were determined. It was suspended with stakes at an average height above the pond bottom of about 5 cm. The tops of the cells rose about 1 cm above the surface of the water. Parthenogenetic female D. magna were placed

individually in cells, and were then observed four to five times daily. The females came from several different source ponds, because of the difficulty, at that time, of finding females still reproducing parthenogenetically in any single pond. Observation times were dawn (about 07:00), 10:00, 14:00, 18:00, and dusk (about 20:00). During the observation times individuals that had released a brood were noted, and were removed later in the day when the new brood had been deposited in the brood chamber. The period between these two points was considered to be the time during which the sex of the newly deposited brood was determined. The female was returned to the lab, and her brood was counted and sexed when released from the brood chamber. At each observation time a measure of water temperature (at a depth of 5 cm), and pH (at a depth of 5 cm) were taken. Temperature was measured with a Yellow Springs Instrument Co. temperature/conductivity/salinity meter (model 33) to an accuracy of 0.5°C. Pond water pH was measured with an Orion Reasearch Inc. Specific Ion Meter (Model 407A) to an accuracy of 0.1 pH units. Records of solar irradiance were measured in integrated periods of 1/2h with a Licor Solar Monitor (Model LI-1776) and the terrestrial Quantum Sensor. An integrated reading of zero E/m2 was considered darkness.

Sex-Ratio Control: Spatial Heterogeneity Experiment

This experiment ran from August 11 to August 26 in 1985. Dip-net samples were drawn each day from clumped and dispersed areas of Daphnia density in the ponds. The distinction between these two densities was a subjective visual one, but was generally quite obvious. Samples were collected from ponds A30, A31A, and A23 successively over the sampling period as females carrying parthenogenetic embryos became rare in each of the populations. The data were pooled across ponds. The two samples were drawn one immediately after the other each day, and returned to the lab where parthenogenetic females were subsampled, and isolated in 100-ml plastic cups of filtered pond water. The cups were kept inside to shorten the incubation period. On release of the brood the offspring were counted, and sexed.

RESULTS

Population Secondary Sex Ratios

The temporal sequence of estimates of population proportion of male neonates for the four ponds studied in 1984 is provided in Figure 2. The four ponds showed a similar pattern. Male production was initially low, but the proportion of male neonates then increased dramatically over a one-to-two week period. The timing of this increase depended upon the pond. The increase in proportion of males did not continue unabated, in each pond (except pond 29) later values appear to equilibrate near 0.5.

Sampling of pond 28 was terminated on July 15. On August 14 the pond dried completely, until August 20 when heavy rains refilled it. The September estimate of neonate sex ratio represents the broods of females (22 females, 339 offspring; three days of brood releases are represented by the single point) that hatched from ephippia after the pond was refilled by a heavy rainfall. Every attempt was made to ensure that the new population was exhaustively sampled. Thus, the September ratio represents a population value, and not a sample estimate. The proportion of male neonates in this sample is consistent with estimates from the same time period for ponds 31 and 28A. The observation of a sex ratio of approximately 0.5 in this population is particularily noteworthy, because conditions were very

Figure 2. Proportion of parthenogenetic male offspring (male offspring / total offspring) produced by Daphnia magna females isolated from four ponds. Each point is the pooled proportion from three days of brood releases. Vertical bars are 95% confidence intervals for sample estimates of a population proportion.

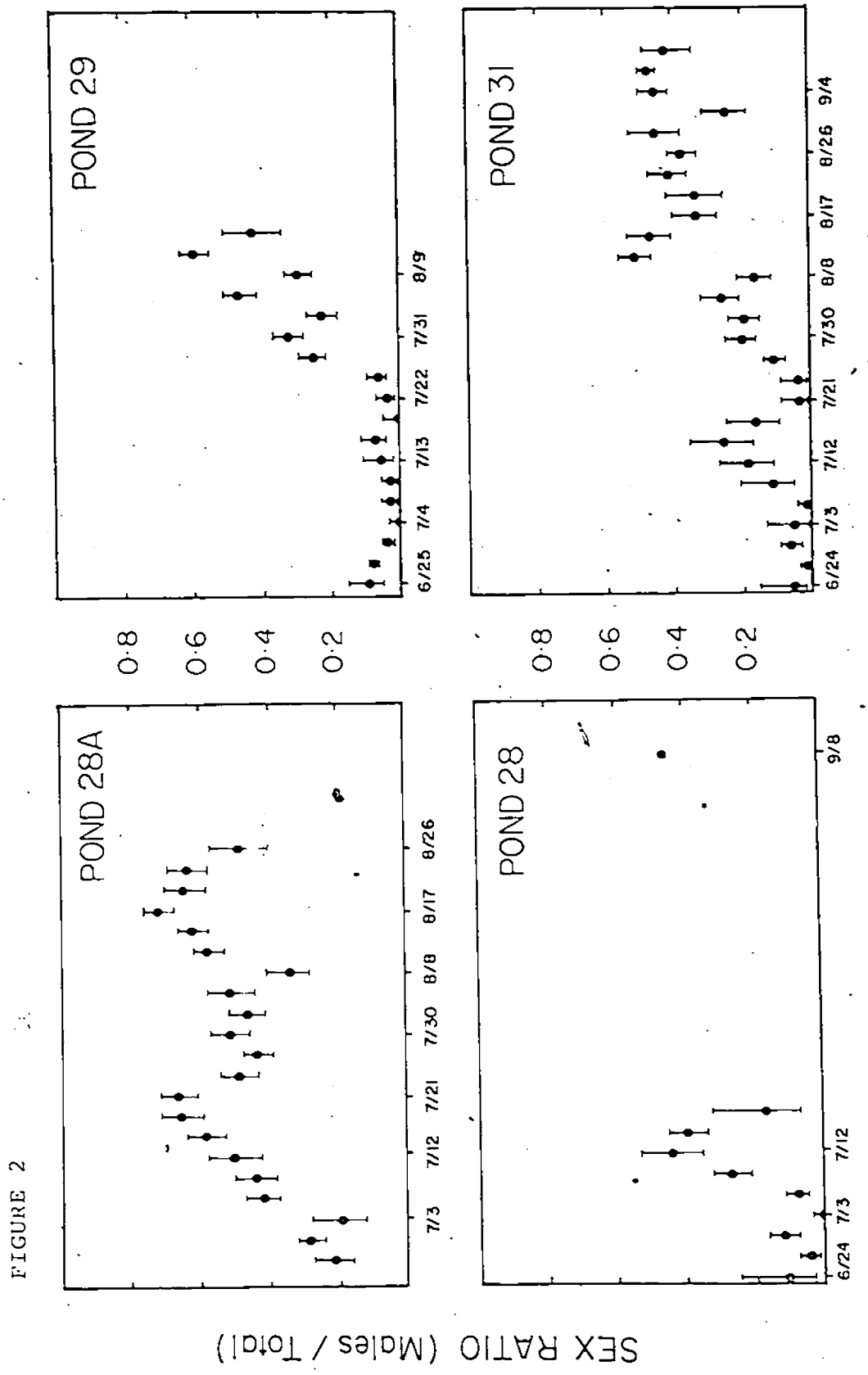


FIGURE 2

different from those in the other ponds (eg. lower density, ex-ephippial individuals).

The data from pond 28A provide a record of neonate sex ratios throughout the entire period of parthenogenetic egg production, since females in this population produced only ephippial eggs after about August 20. Pond survey samples from pond 28A show the proportion of parthenogenetic females among adult females to have been below 1% after August 21 (see Appendix II). Data collection for pond 29 ended due to the mortality incurred when the pond nearly dried on August 14. Many females in pond 31 were still producing parthenogenetic eggs on September 10 when all sampling was terminated.

Juvenile Sex Ratio

Complete results for the 1984 pond survey appear in Appendix II for reference. The pond survey provided information on the proportion of male juveniles among all juveniles counted (Figure 3). This is a tertiary sex ratio, because pre-reproductive individuals of all age classes were combined, and all individuals were past the period of parental investment. The survey samples may be divided into three arbitrary groups on the basis of sampling period (August 1 to 16, August 20 to 28, and September 5 to 10). The juvenile sex-ratio estimates were

Figure 3. Estimates of proportions of juvenile males among juveniles from pond samples of Daphnia magna collected in August and September of 1984. Vertical bars are 95% confidence intervals for sample estimates of a population proportion.

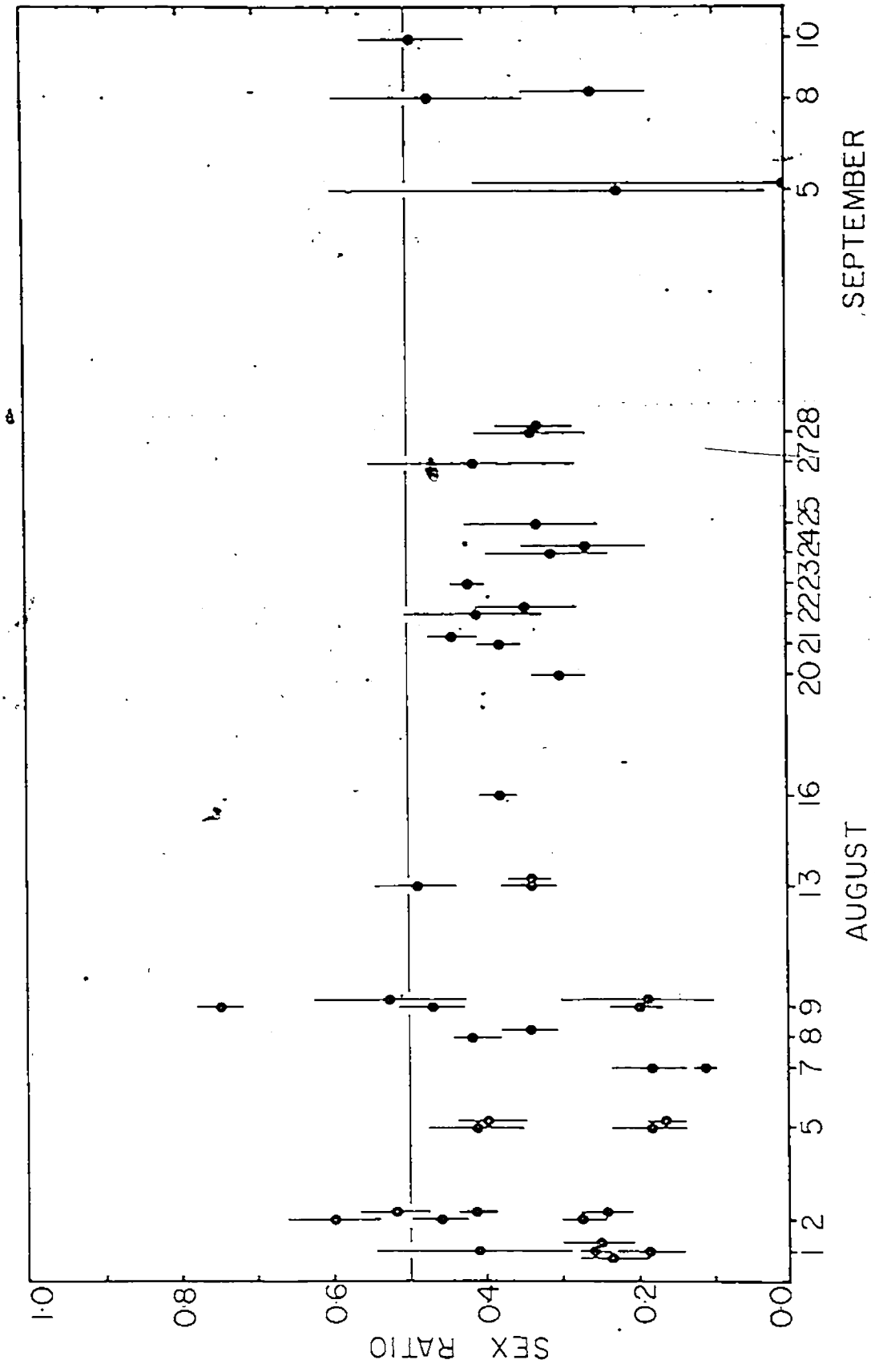


FIGURE 3

transformed (arcsine square root transform), and means and variances were calculated for each group. The variances are marginally heterogeneous (Fmax test, $0.01 < p < 0.05$). ANOVA shows that the means are not significantly different ($p < .05$). A test of unplanned pairwise comparison of means under the assumption of heterogeneous variances (Sokal and Rohlf 1981, p 409) found no significant differences at the $p = 0.05$ level. The grand mean for the entire sampling period was 0.33 (back transformed value). That the means are not significantly different suggests that there was no change over the sampling period in the mean juvenile sex ratio among the ponds sampled. Most of the sample estimates were significantly less than 0.5, but only one estimate (for pond CH-H on August 9) is significantly greater than 0.5. There were changes within individual ponds over time, and examples of such changes are illustrated in Figure 4 for ponds 29 and 31. The survey samples for ponds 29 and 31 spanned the period during which male production began to increase in those populations, and the estimates of juvenile sex ratio reflect this increase.

Relative Expenditure on Male and Female Offspring

If the average allotment of resources needed to produce a male egg is less than that needed to produce a female egg, then male broods should be numerically larger

Figure 4. A reproduction of Figure 2 including the juvenile sex-ratio estimates for these four ponds from the pond survey of 1984. The juvenile sex-ratio estimates are represented by triangles. Dates are labelled only for the collection times of the juvenile sex-ratio estimates. Vertical bars are 95% confidence intervals for sample estimates of a population proportion.

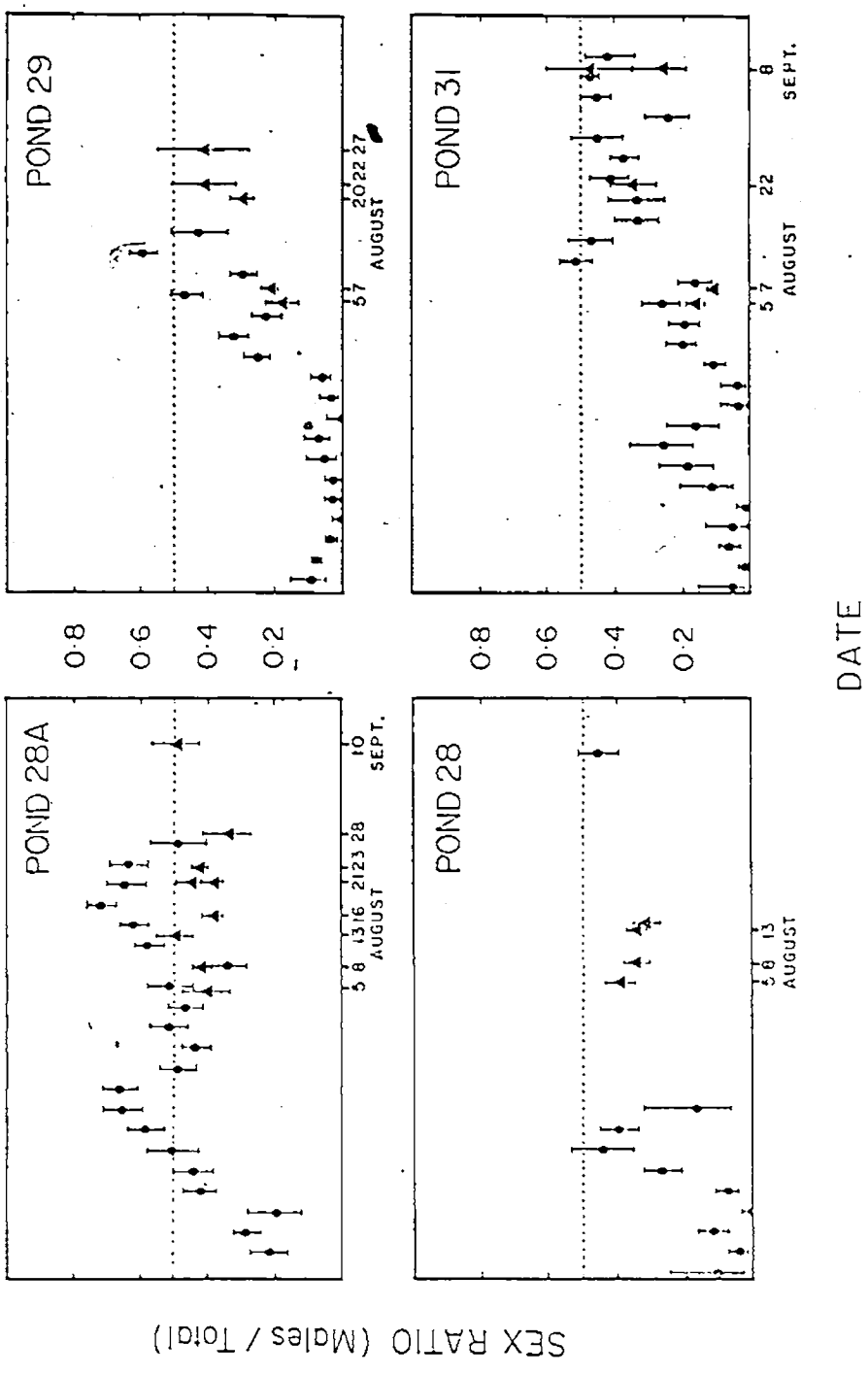


FIGURE 4

than female broods. If males cost the same as females, then the brood sizes should, on average, be equal, and the equilibrium PSAR is equal to the ratio of numbers of male and female offspring. The equilibrium sex ratio is expressed in terms of units of parental investment, but our measure of sex ratio is in terms of numbers of offspring.

To test for the presence of differential investment in the sexes I performed a two-way, mixed-model ANOVA of brood sex and date of brood release on brood size for each pond (Table 1). In all four ANOVA's the date of brood release (DAY) had a significant effect on brood size. This was to be expected, because the DAY variable represents a composite of factors, one of which, food availability, has a major effect on brood size. In all four ponds, brood sex had no effect on brood size. This result is important, because it means that counting numbers of offspring is an accurate estimate of the investment sex ratio for these populations. In two ponds there was a significant effect of interaction between date of release and brood sex ($0.01 < p < 0.05$). This interaction is not unexpected, because the DAY variable represents the effect of time, as well as food availability, on brood size. Food availability is expected to be highest, and broods largest at the beginning of the season, when brood sexes tend to be mostly female. The important result remains that male and female brood

Table 1. Values of p > F from four two-way ANOVAs of sampling day (DAY), and brood sex (SEX) on brood size

	p > F			
Source	Pond 31	Pond 28A	Pond 29	Pond 28
Day	.0001	.0001	.0001	.0001
Sex	.37	.40	.19	.52
Day*Sex	.01	.76	.03	.97

Note: In these models DAY is treated as a random effect, SEX is treated as a fixed effect; F-ratios were calculated accordingly.

sizes are similar when averaged over the season.

Brood Composition

Although Figure 2 shows that the populations began to divide resources between male and female offspring, examination of individual brood compositions indicates that most were single sexed (Tables 2, and 3). Mixed broods constituted 7.6%, and 7.7% of the total number of broods observed in 1984, and 1985 respectively.

The composition of those mixed broods (combined data from 1984-85) is illustrated in Figure 5. The most frequently observed class of mixed brood were broods with a single male individual, followed by broods with two male individuals. In two cases broods containing a single female individual were the second most frequent class observed among broods of a given size, or size range. Broods with a single female were never as frequent as broods with a single male individual.

Brood Sequence: 1984 Data

A summary of the brood series of individual females obtained in 1984 appears in Table 4. The data are arranged in a two-way contingency table for sex of first brood, and sex of second brood. The counts in each cell represent individual mothers that produced a particular sequence. If either the first or second brood was a mixed brood that

Table 2. Sample sizes for each pond sampled in 1984 with a breakdown into the sex of broods (male, female, or mixed)

	Number of Broods			
Pond	Male	Female	Mixed	Total
31	298	1027	88	1412
28A	497	462	105	1064
29	141	714	63	918
28	56	285	28	369
SUM	992	2488	284	3764

Table 3. Sample sizes for each pond in 1985 with a breakdown into the sex of broods (male, female, or mixed)

	Number of Broods			
Pond	Male	Female	Mixed	Total
A28	74	28	10	112
A27	26	115	14	155
A30	153	74	7	234
A31A	172	233	43	448
A23	61	42	8	111
SUM	486	492	82	1060

Note: The sex of all broods was induced in the pond environment. A28 females were collected for the brood sequence experiment, all on August 10. A27 females were collected for the same experiment all on August 12. The broods from A30, A31A, and A23 were collected for the density experiment from August 10 to 24.

Figure 5. Composition of mixed broods. Each histogram shows the number of mixed broods composed of one to n males (and n to one females) among broods of n+1 offspring. For example, the top rightmost histogram includes only broods of eight offspring, twenty-four of those broods had one male, and seven females. The bottom right histogram shows the number of broods which fell within a given range of proportions of male offspring for broods of twelve to twenty-five offspring. Combined data on mixed broods from 1984 and 1985 are included in the figure.

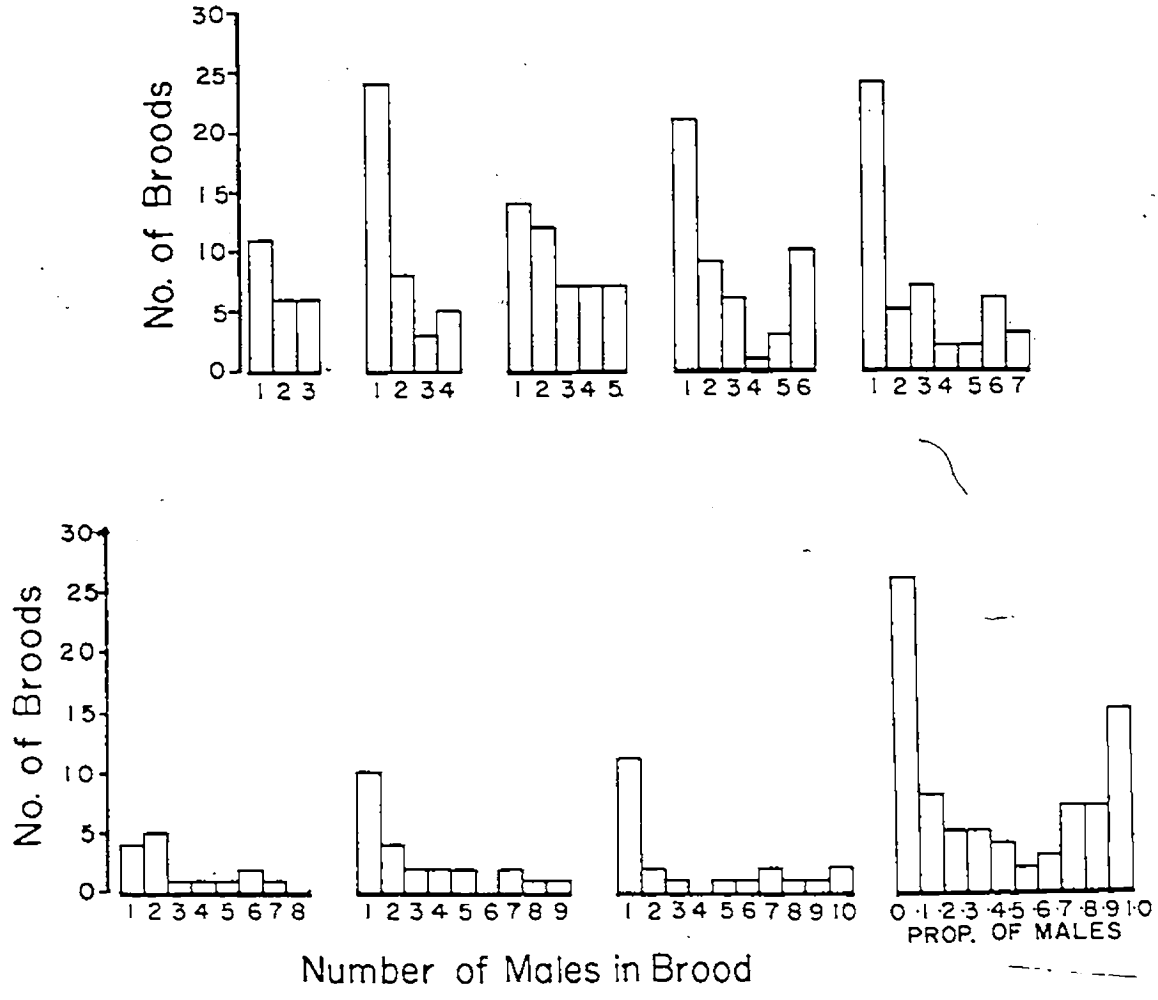


FIGURE 5

Table 4. Numbers of broods from four brood sequence pairs, 1984 data.

		Brood Two		
		Male	Female	Sum
Brood One	Male	62	110	172
	Female	20	29	49
	Sum	82	139	221

Note: Mixed sex broods are excluded from the table. The data are arranged as a two-way contingency table representing the four possible brood sex sequences obtainable when considering two successive unisexual broods. For these data; $\chi^2 = 0.36$, $p > 0.05$, $df = 1$

mother was excluded from the analysis. A chi-square test shows that the sex of the second brood was independent of the sex of the first brood. The sex ratio among first broods (which is male biased due to investigator bias) is 0.78 (95% confidence interval = 0.833 to 0.731). The sex ratio among second broods is 0.37 (95% confidence interval = 0.425 to 0.317). Of the original (22%) female producers, 60% remained as such, 40% became male producers, and of the original (78%) male producers, 36% remained as such, 64% became female producers. The second brood was more likely to be female than male, regardless of the sex of the first brood.

Sex-Ratio Control: Endogenous Cycle Experiment

This experiment provided data on the size and sex of two to five successive broods of individual mothers. The experiment was designed to test for a strict alternation of brood sex by individual mothers (i.e. MFMFMF...). The 1984 brood-series data (Table 4) falsifies this hypothesis since all sequences were found to be of equal likelihood, when sequences of MF and FM should have been produced exclusively. Table 5 tabulates the first two broods from the brood-series of females observed in 1985. The first brood of the series was determined for sex in the pond, the second brood's sex was determined in the cup environment,

Table 5. Number of broods from four brood sequence pairs in the endogenous cycle experiment of 1985

		Brood Two		
		Male	Female	Sum
Brood One	Male	11	6	17
	Female	14	55	69
	Sum	25	61	86

Note: Mixed sex broods are excluded from the table. The data are from the first two broods of the endogenous cycle experiment. The data are arranged as a two-way contingency table representing the four possible brood sequences obtainable when considering two successive unisexual broods. For these data $\chi^2 = 13.4$, $p < 0.001$, $df = 1$.

as in the 1984 observations. The chi-square value (χ^2 = 13.4, df=1) shows that the sex of the second brood was not independent of the sex of the first brood. The sequences MM and FF were more frequently observed than would be expected on an assumption of independence. The experimental hypothesis is falsified by this second set of data also. It is of interest to note that the observations from 1985 differ from those of 1984. In the 1985 experiment a female brood was only likely to be produced if a female brood was produced first.

The complete brood sequence data is summarized in Table 6. Two qualitative observations may be made at this point; first, there is an apparent tendency for a female to continue producing male broods once a male brood has been produced. Secondly, females tend to produce broods of only one sex, and not both in equal numbers, or as near to equal numbers as the sample allows.

Some of the mothers produced ephippia during, or at the end of, their brood series. Of those that did so (n = 50), 14 produced the ephippium after a male brood, and 36 produced an ephippium after a female brood. The overall proportion of male broods in the experiment was 0.38 (see the data in Appendix IV). The production of an ephippium did not follow a male brood any more, or less, frequently than a female brood (χ^2 = 0.42, df = 1, the overall ratio

Table 6. Summary of brood sequence data from the 1985 endogenous cycle experiment.

Number of Broods in Sequence							
2		3		4		5	
SEQ	N	SEQ	N	SEQ*	N	SEQ*	N
MM	5	MMM	2	MMMM	2	MMMMM	2
		MMF	0	MFMM	1	MFMMM	1
MF	3	MFM	1	FMMM	1	FMMMM	3
		FMM	3	FFMM	1	FFMMM	1
FM	5	MFF	0	FFFM	3	FFFFM	1
		FMF	2	FFFF	11	FFFFF	5
FF	26	FFM	0				
		FFF	9				
SUM	39		17		19		13

Note: Number of females (N) which produced a brood series of given length and sequence. '*' indicates that only those brood sequences observed are listed. In series of four broods there are 16 sequence possibilities, in series of five broods there are 32 sequence possibilities. Females counted in brood series of two broods produced only two broods during the experiment, and then either died or produced an ephippium. Likewise for the other series lengths.

of male to female broods was used as an extrinsic hypothesis in this test).

The data from this experiment exhibit two other patterns which are worth mentioning. Figure 6 (raw data in Appendix IV) shows that with each successive brood the experimental females produced, the proportion of male broods increased. Figure 7 (raw data in Appendix V) shows a decrease in mean brood size with each successive brood. The data suggest that the females were food limited throughout the experiment. Although brood size and proportion of male broods appear to be correlated, male broods were not smaller than female broods (Figure 8).

Sex-Ratio Control: Temporal Cycle Experiment

This experiment provided data on sex of brood and time of brood release during the day. Observations were also made on diurnal cycles of photoperiod, pond temperature, and pond pH. All of the data are summarized in Figure 9. At the base of the figure the periods during which sex was determined (Banta and Brown's 1929 critical period) for twenty broods are marked. The number of broods is too small to draw convincing conclusions. The variables measured each divide the day into two parts; high or low temperature and pH, increasing or decreasing temperature and pH, and periods of light and dark. Brood sex (male or female) does not appear to be related absolutely to any of

Figure 6. Proportion of male broods in successive broods of females during the 1985 endogenous cycle experiment. Every female is included in the estimates. If a female only produced two (to four) broods, and died or produced ephippial broods afterwards she was included in the first two (to four) estimates of brood sex ratio. An ephippium was counted as a brood but did not figure in the estimates for sex ratio. Mixed broods were excluded from the analysis. Vertical bars are 95% confidence intervals for sample estimates of a population proportion.

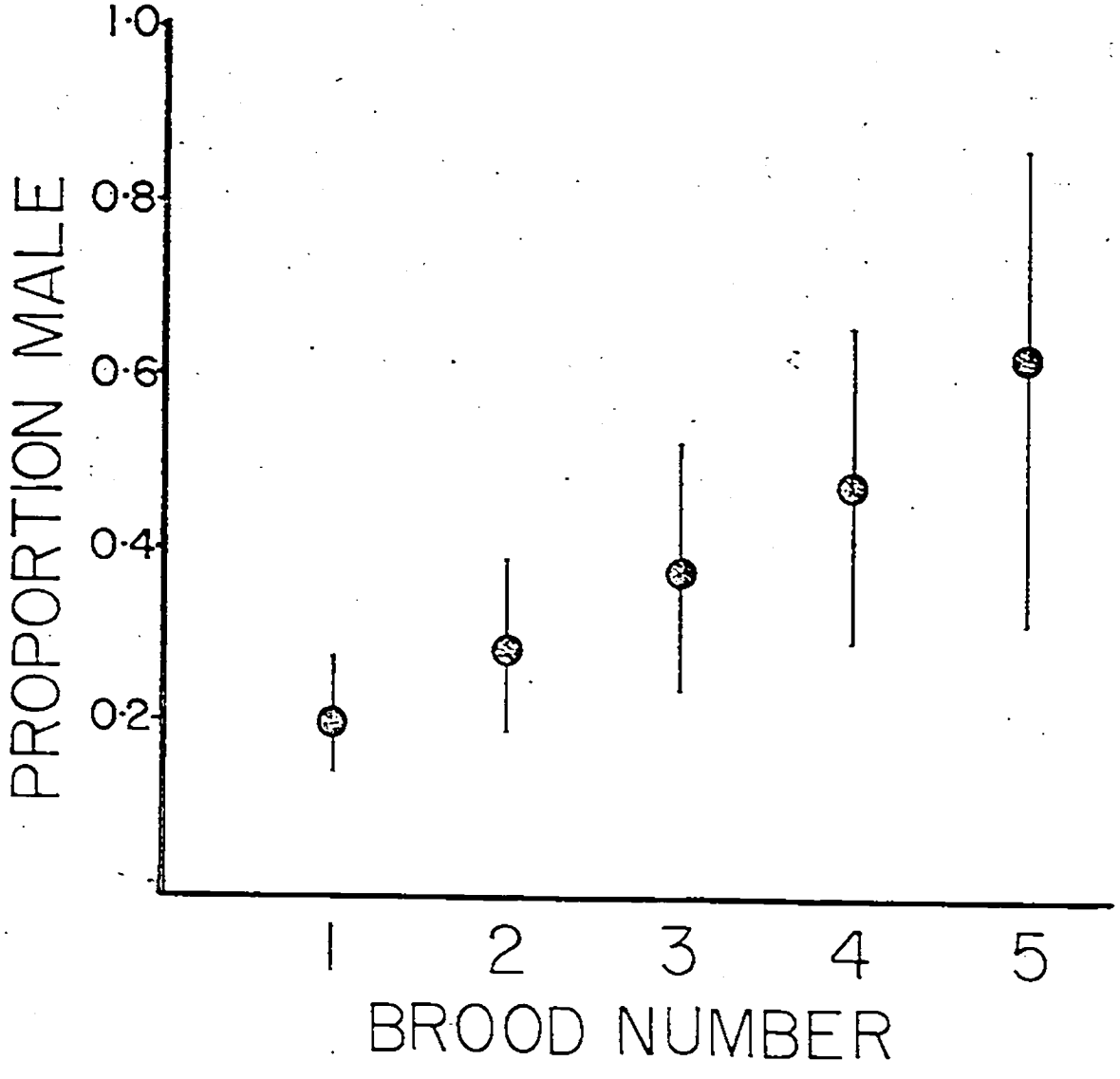


FIGURE 6

Figure 7. Average brood size for successive broods of females during the 1985 endogenous cycle experiment. Brood number is decided according to the rules outlines for Figure 6. Mixed broods were included in the analysis. Vertical bars are 95% confidence intervals for sample estimates of population means.

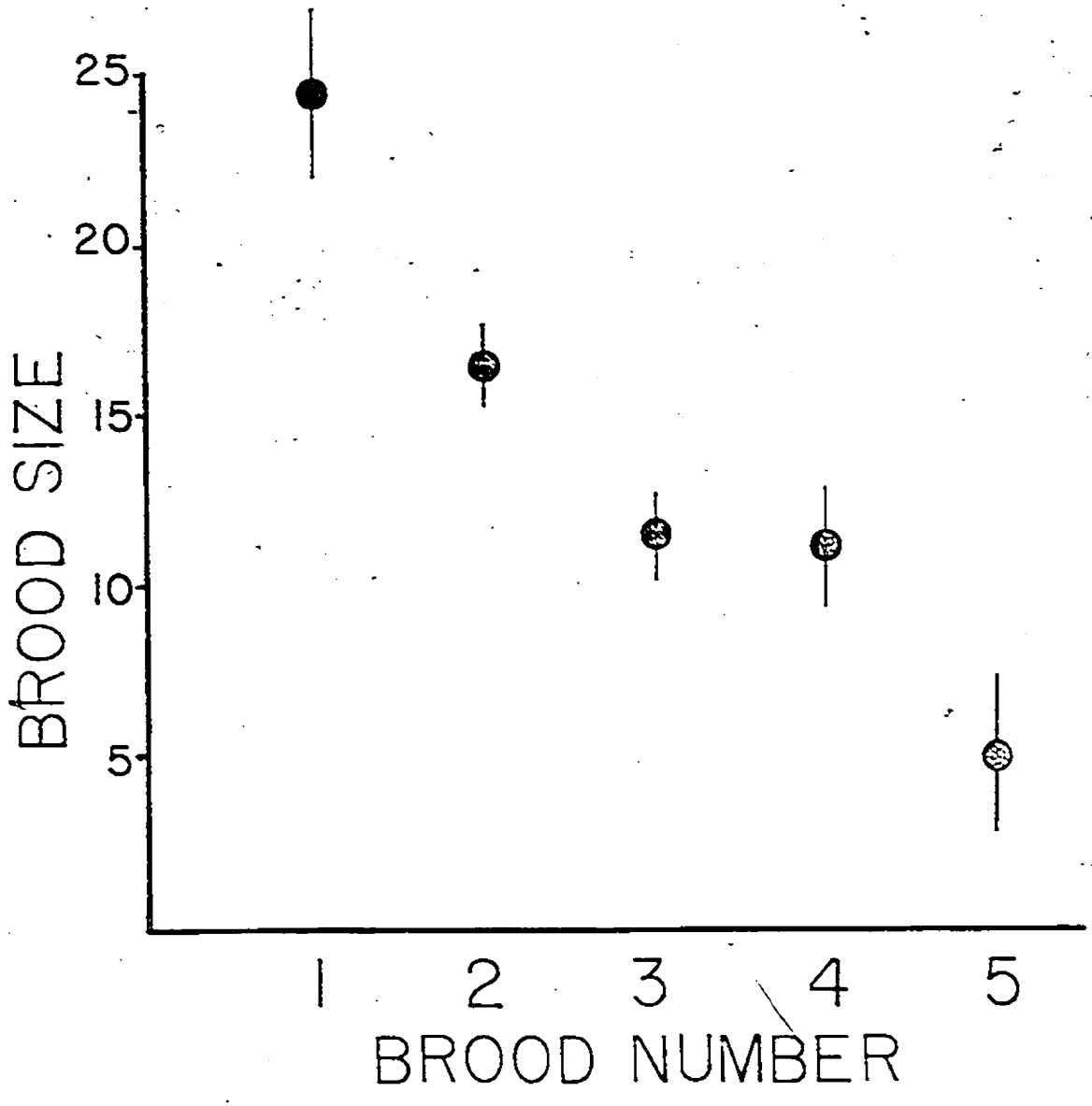


FIGURE 7

Figure 8. Average male and female brood size for successive broods in the endogenous cycle experiment of 1985. The figure divides the data of figure 7 (and Appendix V) into male and female broods. Mixed broods are excluded from the analysis. Vertical bars are 95% confidence intervals for sample estimates of population means.

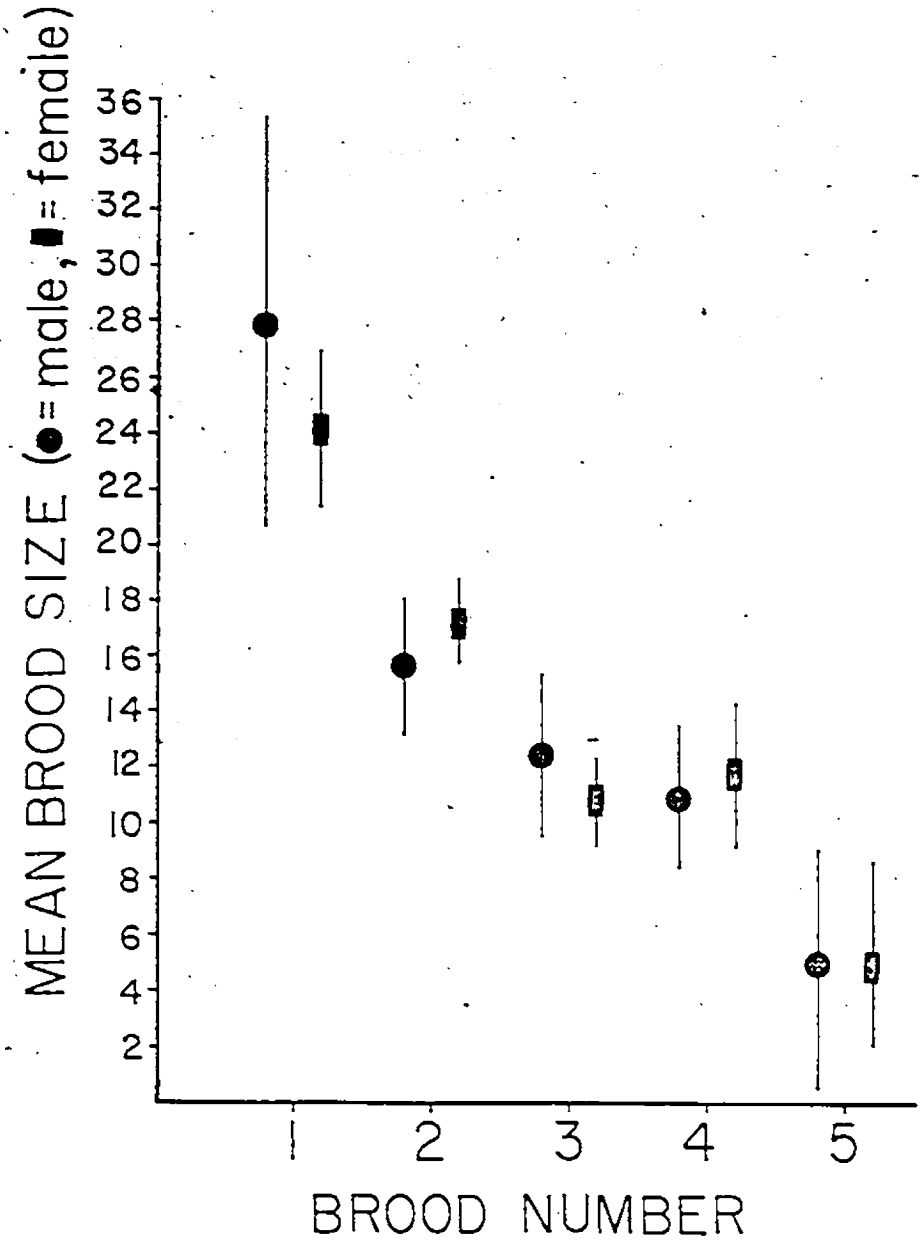


FIGURE 8

Figure 9. Composite of data gathered from the temporal cycle experiment. The upper portion of the figure is divided along the horizontal axis into 24h days (the line passes through midnight). The bold line curve interpolates pond temperature readings (open circles). The light line interpolates pond pH readings (solid circles). Immediately below, periods of light and dark (photoperiod) deduced from the integrated solar irradiance readings are shown. Below this, arrows indicate periods of increasing and decreasing pH and temperature. And finally the periods during which brood sex was determined for male, female, and mixed broods are marked as bars. Each bar represents a single brood.

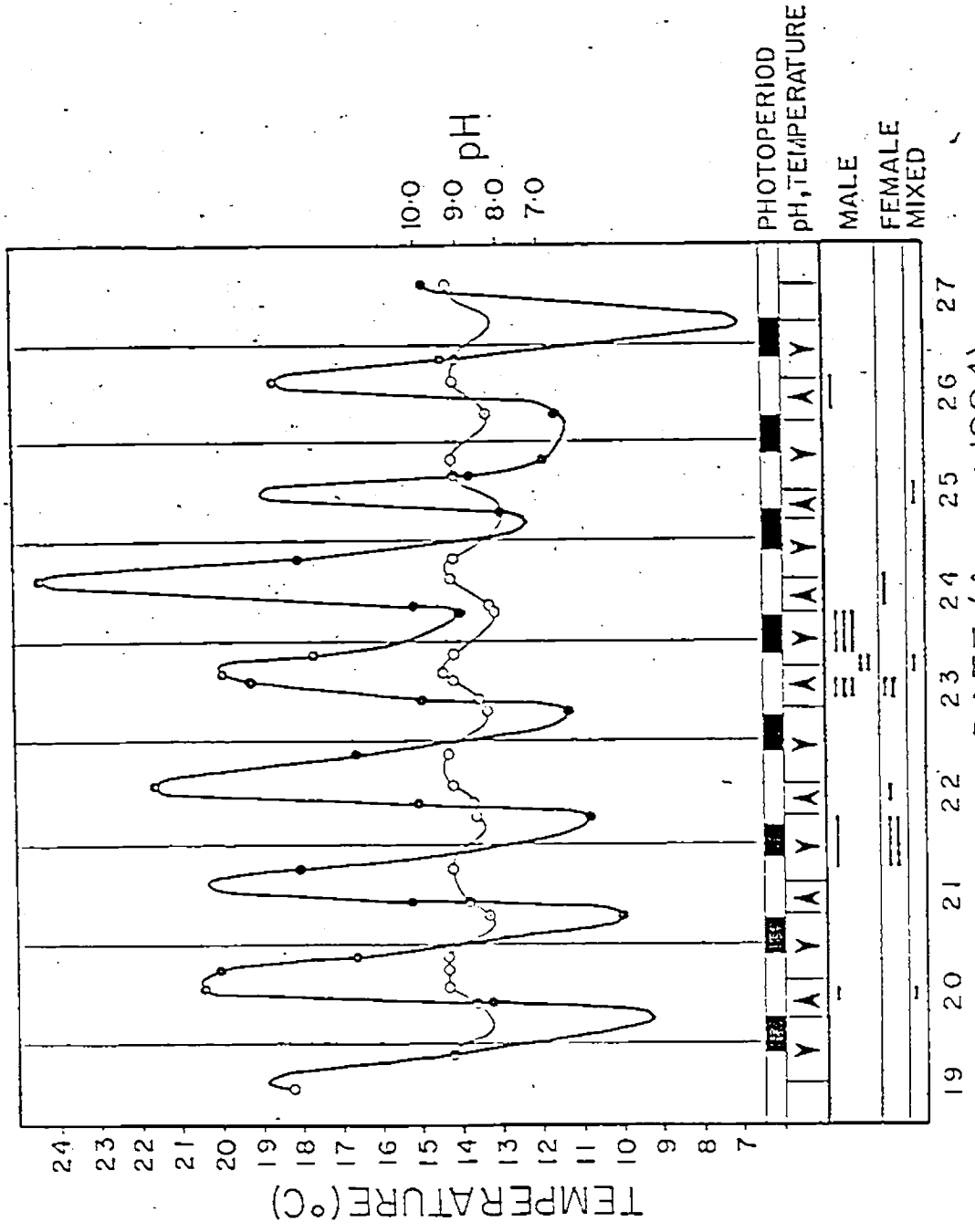


FIGURE 9

these periods. Five male broods were found from periods of increasing temperature and pH, four female broods were found from the same conditions. Four male broods were found from periods of decreasing temperature and pH, two female broods were found from the same conditions. Two male broods include both increasing and decreasing periods (and a temperature and pH maximum). Of the mixed broods, one was found from a period of increasing temperature and pH, and the other two from a period spanning an increase and a decrease of temperature and pH (both cover maxima of temperature and pH). Similar results are obtained when periods of light and dark are related to brood sex.

Sex-Ratio Control: Spatial Heterogeneity Experiment

The final sex-ratio control experiment provided data on the sexes of broods produced by females collected from areas in a pond of high and low Daphnia density. The data were collected over a number of days with collections made on each day. Because the hypothesis being tested is only that sex ratio should differ between the two density treatments, the data from different days' collections have been pooled across days. The females released their broods within four days of collection. The combined data from all four days of brood releases are presented in Table 7. If density is the only factor affecting the sex of a brood we

Table 7. Number of male and female broods from females collected in areas of high and low *Daphnia* density, 1985

		Brood Sex		
		Male	Female	Sum
Density	High	2076 (2133.8)	2329 (2271.2)	4405
	Low	1312 (1254.2)	1277 (1334.8)	2589
	Sum	3388	3596	6994

Note: The data are from the 1985 spatial heterogeneity experiment. They are arranged in a two-way contingency table. Numbers in parentheses are expected values. For these data $\chi^2 = 8.20$, $p < 0.05$, df = 1.

expect to find an absolute interaction between brood sex and density treatment; all broods coming from females collected in high density areas will be of one sex; and all broods from females collected at low density will be of the other sex. Brood sex was, indeed, not found to be independent of density treatment, and the deviations show that there were fewer male broods in high density areas than expected. The interaction between brood sex and density treatment is not strong. This suggests that a factor, or combination of factors, other than density is affecting brood sex. Either the interfering factor is imperfectly correlated with density treatment, or it is a factor masking the effect of density treatment on brood sex. An example of the latter possibility, which we are able to control for to some degree might be the effect of time. The greater the time span after the time that her brood's sex is determined the greater the probability that she has changed her position. Depending on the time scale of the mother's movements we might expect the data from the fourth day of release, alone, to reveal a stronger interaction, since only those females are considered which had most recently had their brood sexes determined when collected. The complete data, subdivided into four days of release appear in Table 8. The chi-square value obtained using only the fourth day's data ($\chi^2 = 14.58$, df = 1) is

Table 8. Numbers of male and female broods released one to four days after females were isolated in the 1985 spatial heterogeneity experiment

	Day 1		Day 2		Day 3		Day 4	
	Male	Female	Male	Female	Male	Female	Male	Female
Density								
High	667	778	736	744	505	508	168 (194.4)	299 (272.6)
Low	359	494	461	329	324	282	168 (141.6)	172 (198.4)

Note: The data come from the 1985 spatial heterogeneity experiment. Only the Day 4 data are discussed in detail in the text, for that subset of data; test of independence of brood sex and density treatment, $\chi^2 = 14.58$, $p < 0.05$, $df = 1$. Parentheses contain expected values based only upon the Day 4 subset.

higher than the value obtained for the pooled data, and the deviations are in the same direction (fewer male broods from high density areas than expected). The results do not indicate an absolute response of brood sex to density treatment, but they are suggestive.

DISCUSSION

Population SSR: *Daphnia magna*

The present study has shown production of approximately equal numbers of male and female offspring during the main period of male production in the Churchill populations. The observations on pond 28A, in particular, suggest that the proportion of male offspring produced does not, within the range of error detectable in our samples, increase beyond 0.5 at any time during the season. Pond 31 corroborates this conclusion since, although parthenogenetic reproduction had not stopped, low temperatures after September 4th would have limited the reproductive contributions of the remaining population (mean daily air temperature for September was 4.1°C (Churchill Weather Office summary)).

Population SSR in Other Cladocerans

Comparative data from other cladocerans and other populations of *D. magna* suggest that the pattern observed in the study populations might be general. Banta (1937) reported that he had never observed 100% male production in natural populations of *Moina spp*. He speculated that the average maximum of 40% male production observed under diverse conditions in laboratory experiments on male induction in *Moina* might approximate male production maxima

in nature. In a recent study Korpelainen (1985) describes lifetime neonate sex ratios in clones of Daphnia magna under varied treatments of photoperiod, and temperature. Both factors affected offspring sex ratio, and the range of treatments included the normal range of conditions experienced by the source populations in nature. Korpelainen found a peak incidence of 50% male offspring. This result is not unusual in laboratory studies of male induction in the Cladocera. These studies only rarely report neonate sex ratios significantly greater than 0.5 (Banta et al. 1939, Leary 1967, Stimpfl 1971, and Ferrari and Hebert 1982). Another recent study (Schwartz and Hebert, 1986a) examined neonate sex ratios of field-collected female Daphniopsis ephemeralis. The species occurs for brief periods in vernal and autumnal ponds. In most populations the sex ratio among first broods of ex-ephippial females was not significantly different from 0.5. In this species the period of female-only parthenogenesis has been eliminated, producing a sex-allocation pattern resembling the behaviour, during the latter part of the season, of the arctic D. magna populations of this study. Indeed, Schwartz and Hebert (1986b) report the occurrence of ex-ephippial males in D. ephemeralis, which represents yet another step in the elimination of a female-biased sex ratio. These results corroborate our observations on

Daphnia magna, and suggest that there may be some generality to male-production limits in cladoceran populations.

Population SSR in Aphids

This section examines the available cytogenetic, and sex-ratio data on the aphids. The Aphidoidea (which are all either cyclic, or derived obligate, parthenogens (Bell 1982)) are one of four superfamilies in the suborder Sternorrhyncha of the insect order Homoptera. The superfamily consists of four families, the Aphididae (aphids), the Eriosomatidae (wooly aphids), the Chermidae (pine and spruce aphids), and the Phylloxeridae (Borror et al. 1976). There are two reasons for comparing the present results with information from studies of aphids. First, the aphid life history is very similar to that of the cladocerans, a fact that has often generated comparative discussions (Morgan 1909a, Young 1983, Shull 1925 and 1929). Secondly, the mechanism of sex determination in the aphids is understood, and valuable data are available on natural, and laboratory secondary sex ratios. These sex-ratio data are not available for the monogonont rotifers, although certain conclusions from this comparison probably also apply to rotifers.

Morgan's (1909a) study of the chromosome cycles and life histories of two phylloxerans (Phylloxera fallax and

P. caryaecaulis), which form galls on hickory, was responsible for the view that environmental conditions controlled the appearance of sexual forms (males and sexual females), but that sex was determined genetically. In P. fallax a stem-mother hatches from an overwintering egg, and begins to feed from a vein on the underside of a hickory leaf. Her feeding induces gall formation, and in time the gall completely encloses her. She lays parthenogenetic eggs which hatch into females that are either winged (alate), or wingless (apterous). These female offspring give rise to the sexual generation, one type of female exclusively produces males, another type exclusively produces females. There is no chromosomal difference between these two types of female. The eggs giving rise to the sexual generation are laid within the gall, which only rarely breaks in time for their mothers to escape. If the gall does break some winged individuals, which are invariably male producers, may escape to lay their eggs outside of the gall. The male eggs are smaller than the female eggs. The males and females leave the gall after they hatch, pair, and the females deposit their eggs on the stems of a branch on their hickory tree. These eggs overwinter, and hatch into stem-mothers the following spring.

The life history of P. caryaecaulis is similar to that of P. fallax in general outline. However, the second

generation is made up only of winged individuals which leave the gall to deposit male and female eggs on the undersurface of the hickory leaves. The winged generation consists of male producers and female producers, and the male egg is smaller than the female egg as in P. fallax.

The chromosome cycle of P. fallax is representative, in outline, of the entire group (the order Aphidoidae, as far as is known (White 1973)). Eggs produced by the stem-mother all contain 12 chromosomes, but the eggs produced by the second generation contain either 10 or 12 chromosomes, depending on whether they will develop as males, or females, respectively. The timing of the maturation divisions of the aphid and cladoceran parthenogenetic egg are similar. The division begins before egg laying, and is completed, with the release of the polar body, after laying. During the maturation division of the male eggs two of the twelve chromosomes pass into the polar body whole, rather than dividing like the others. These two chromosomes leave behind their homologues. Altogether there are four of these accessory, or sex, chromosomes in two pairs. Males are therefore 'X_1X_200' and females are '$X_1X_2X_1X_2$'. Each spermatogenesis event in the male results in only two functional spermatozoa. In meiosis I the two accessory chromosomes pass to one of the spermatocytes, and the other spermatocyte degenerates. In meiosis II the

accessory chromosomes divide like the other chromosomes in a normal reduction division. Upon fertilization the two accessories in the male are reunited with their homologues in the egg, thus giving rise only to female offspring. Female oogenesis is of the normal kind, resulting in a single egg with six chromosomes.

All aphids and phylloxerans with holocyclic (heterogonic) life cycles that have been examined exhibit an XX-XO sex determining system (White 1973). Not all aphids possess strictly male and female producing individuals (White 1973), and the size dimorphism in male and female eggs is apparently not typical of all phylloxerans (Morgan 1909b).

Morgan reports on natural SSR's in the studies on phylloxerans. These observations are summarized in Table 9. He (Morgan 1909a) also provides information on egg size and egg production, which allows a conversion from egg counts to actual PSAR for the populations. In P. fallax some of the winged individuals may deposit their eggs outside the gall, these individuals apparently produce only males. The number of male-producing emigrants is unknown, though apparently small, and hence we might expect the proportion of males to be slightly greater than the estimates. In P. fallax the male eggs are smaller than female eggs, and hence cost less, per egg, to produce.

Table 9. Summary of Morgan's Secondary Sex Ratio Estimates for Two *Phylloxera* Species

Species	r(obs.)	n	r(exp.)	Data on:	Deviation	Reference
P. fallax	0.496	2116	0.62	F	Low	Morgan 1909b
	0.562	1055	0.62	F	Low	Morgan 1909a
	0.392	1033	0.62	F	Low	Morgan 1915
P. caryaecaulis	0.816	1612	0.67	F	High	Morgan 1909b
	0.737	4043	0.50	P	High	Morgan 1909b
	0.83	---	0.67	F	High	Morgan 1909a
	0.74	6528	0.50	P	High	Morgan 1909a

Note: r(obs) is the estimate of SSR. r(exp) is the expected egg or individual sex ratio if the PSAR = 0.5. Morgan counted male and female eggs (F = filial), and male and female producers (P = parental). All deviations from r(exp) of r(obs) are significant at the p = 0.99 level.

Morgan (1909a) made measurements on the lengths of the major and minor axes of three of each of these eggs, which allow us to roughly estimate their relative costs of production. If the eggs are ellipsoid in form the male egg contains about 0.62 times the volume of the female egg. If the population PSAR were 0.5 the secondary sex ratio should be 0.62, assuming egg volume reflects cost of production. However, the standard deviation of Morgan's measurements are large, and allow the egg-cost ratio for males to range between 1.35 and 0.29 ($p = 0.95$). In P. carvaecaulis Morgan (1909a) estimates that male producers carry, on average, twice as many eggs as female producers (16 vs 8), although he admits there was considerable variation in egg number. As gall size (or resource availability) had little effect on whether an individual became a male or female producer, it can be assumed that the two types are investing equally into their offspring, and that the PSAR is equal to the proportion of male producers found. If males are half as costly as females to produce the proportion of male eggs should be 0.67.

The summary of Table 9 shows consistent trends for the two species. P. fallax was found to produce a sex ratio consistently lower than expected, perhaps reflecting the loss of males due to the migration of their winged mothers from the gall. On the other hand, P. carvaecaulis shows

sex ratios consistently higher than expected, reflecting a bias towards males. The degree of deviation depends on the accuracy and representativeness of Morgan's egg measurements and egg production estimates, and should be considered extremely tentative.

Lees (1959) performed experiments examining the effects of photoperiod and temperature on form production in the aphid Megoura vicia, whose sole host is the broad bean, Vicia faba. At a temperature of 15°C apterous viviparae (or parthenogenetic females; they are called viviparae because the Aphididae give rise to live parthenogenetic offspring) produced viviparae and males in 16h daylengths and oviparae (sexual females) and males in 12h daylengths. Approximately the same numbers of males were produced under each daylength. Mean male proportion in the offspring of seven mothers was 0.11 (659 offspring) at a 16h photoperiod, and 0.14 (664 offspring) for seven mothers at a 12h photoperiod. Males tended to appear in the middle of a female's reproductive period. At the intermediate photoperiod of 14.5h light, all three forms were produced. Male production was found to be influenced by temperature, males were not produced at 25°C, were found in roughly equal percentages at 20°C and 15°C, and were supressed somewhat at 11°C. Lees (1959) examined the question of a sex-ratio control mechanism for he was.

convinced such a mechanism existed. He speculated that both direct environmental effects on the type of maturation division, and physiological control mechanisms on the part of the mother might regulate sex ratio in this species.

A second study (Lees 1960) revealed similar reproductive patterns under a different experimental procedure. The tendency for protandry was again apparent. It was also remarked that sub-fertile females produced fewer males, because of their tendency to restrict male production to the middle of the reproductive period. Food limitation might well be an explanation for the very low sex ratios found in this study.

MacGillivray and Anderson (1964) studied morph production in the aphid Macrosiphum euphorbiae, cultured on the potato Solanum tuberosum. The experiments consisted in maintaining successive generations under given photoperiod and temperature regimes by selecting viviparous females from each generation to continue into the next generation. Alate (winged) viviparae produced very few occasional males. Apterous viviparae produced large numbers of males under certain treatments. The longest photoperiod used was 14h light, but the best data for male production come from experiments conducted in 13.5h light. Two such experiments were run, one at 65°F (18.3°C), and another at 51°F (10.6° C). In the first experiment only viviparae were produced

in the first two generations, in the 3rd, 4th and 5th generations viviparae and males were produced in very nearly equal numbers. In the second experiment fewer males were produced, and a few oviparae were produced. In their experiments temperature appeared to have an effect on production of males, but photoperiod did not. The viviparae are amphigenous, and apparently male production begins after production of the female morphs, not in the middle of the reproductive period as in Megoura vicia. It is not clear whether this is due to a problem with sub-fertility as in Lees' studies. One of the problems with comparing this study with those of Lees is the non-overlapping treatment ranges. One suspects that MacGillivray and Anderson did not observe the full range of reproductive behaviour because of their restricted choice of photoperiods and temperatures. Specifically, had they used a longer photoperiod they might have found viviparae producing both males and oviparae as Lees had.

Mayo and Starks (1972) reported low numbers of males in the aphid Schizaphis gramininum. They sampled 10 times at unspecified periods during the life cycle from a greenhouse population. They collected a total of 371 sexual forms with an average proportion of males of 0.11 (backtransformed mean). The highest proportion of males in a sample was 0.33. The samples were biased to periods of

maximum occurrence of sexual forms.

The degree of diversification in aphids since their adoption of heterogony has been far greater than that found in the Cladocera. Consequently it is difficult to create constructive generalizations of aphid life history, or biology. The aphid SSR's reviewed here probably depend less upon the cyclically parthenogenetic life cycle than forces generated by individual species' life histories. Moreover, the estimates of aphid (vs. phylloxeran) sex ratios are all drawn from laboratory experiments, or unnatural conditions, and so their consistently low values may be artifactual. Aphids differ from the Cladocera in possessing a specialized sexual female, which is not capable of parthenogenetic reproduction. The selected SSR in aphids must be sexual females to males, not females to males as it is in the Cladocera. This specialization has not made aphid sex-ratio adaptations any less complicated. For example, in Lees' (1959) study, viviparae produced males and asexual (viviparous) females, other individuals gave rise to sexual females and males at a later point in time. This uncoupling of the appearance of males and females in the life cycle parallels the cladoceran adjustment in sex ratio over time. Another problem remains common to both aphids and cladocerans (Morgan 1909a, Shull 1929). Despite our understanding of the mechanism of sex determination in

aphids, the factors which determine that mechanism's direction (to production of a male, or female egg) are unknown. It is at this level that sex-ratio control must occur. The same question (a separate though related question to the sex determination mechanism) is of concern in cladoceran studies.

Sex-Ratio Control in Daphnia

At this point some clarification of the meaning of cladoceran ESD is necessary. Daphnia do not fit the Charnov-Bull model for the evolution of ESD in one important respect. In Daphnia offspring sex is determined prior to, or during, release of the egg to the brood chamber. The egg probably does not have access to different information about the environment than its mother at this time, because the egg is confined to the maternal body. There is no apparent reason (under the presently existing sequence) for the mother to relinquish control over her offspring's sex. This would be required under the Charnov-Bull model for the evolution of ESD to have taken place. The fact that sex is determined while the egg is in, or leaving, the ovary, and not sometime afterward suggests that the mother is exercising the control of sex. In all other ESD species that have been studied sex is determined, by the offspring, after it is separated spatially and physiologically from the mother (Bull 1983).

A more likely scenerio is a direct parallel to the aphid mechanism of sex control, in which the appearance of males is environmentally stimulated, but sex determination is effected through a mechanism of facultative sex-ratio manipulation. How the mother controls the sex of the offspring she produces is not understood in the aphids, or in the cladocerans. The problem was approached experimentally in this study with the three experiments on sex-ratio control. The experiments provided no clear answer. The endogenous-cycle experiment showed some tendencies in sex-allocation patterns of the mothers, but did not show a simple pattern suggesting that sex determination of individual broods is free from environmental influences (i.e. controlled by maternal physiological cycles). The temporal cycle experiment showed no correlation between diurnal cycles in the pond and brood sex, but the data set is small. The spatial heterogeneity experiment showed some association between Daphnia density and brood sex. A more absolute response may have been obscured by the history of the females' movements between determination of their broods' sexes and their capture.

Daphnia Sex Determination

The introduction to this study outlined the debate

over sex-determining systems in the Cladocera. It is not clear that a chromosomal sex-determining mechanism has been adequately ruled out in the Cladocera. Every study of cladoceran cytology comments upon the difficulty of visualizing the chromosomes, and arriving at accurate estimates of chromosome number (Allen 1928, Allen and Banta 1929, Lumer 1937, Ojima 1958, Bacci et al. 1961). The size of the chromosomes would make it potentially very difficult to detect the lack of a single chromosome in an XO sex determining system, or the presence of a small Y-chromosome in an XY system. On the other hand, even though sex may not be truly environmentally controlled it is not necessary to prove a chromosomal sex-determining mechanism. The mother may be exercising control over sex through physiological interactions between her body and the maturing oocyte. This may be what is happening to the maturing aphid oocyte; maternal physiology leads to a male or female producing maturation division. In the Cladocera the mother's physiology may go a step beyond affecting chromosome behaviour during this division to affecting the expression of the (intact) genome after the maturation division. This is the view of Gilbert and Williamson (1983) who state that "environment presumably controls the sex of an individual indirectly, through the maternal physiology. Bull (1983) notes the widespread laboratory

phenomenon of hormonal control of sex expression (experiments in which sex is reversed by treatment with sex hormones). Detailed sex-reversal experiments have been performed on selected crustaceans (Charniaux-Cotton 1965). External influences of hormones on sex determination are unknown in natural systems (Bull 1983). However, male sex determination in Bonellia viridis (a marine polychaete worm in which larvae develop as female if isolated, or male if they settle upon a female) appears to be controlled by chemical compounds released by the female host (Crew 1965). Other similar examples in which a chemical effect has not been shown would be Ione thoracica, an isopod parasite on fish gills (Crew 1965), and Stegophryxus hyptius, an isopod parasite of hermit crabs (Reinhard 1949). If cladoceran sex was hormonally controlled by the mother, without the intervention of a chromosomal sex-determining mechanism, we would have a unique study system. Sex-ratio control would stand one step further along the line of causation; a mechanism in which maternal physiology was controlled in some way to lead to an adaptive ratio of offspring sexes.

In summary the results of this study describe a characteristic temporal sex-allocation pattern for these Daphnia magna populations. Limited data from other species suggest that this pattern may be shared by other

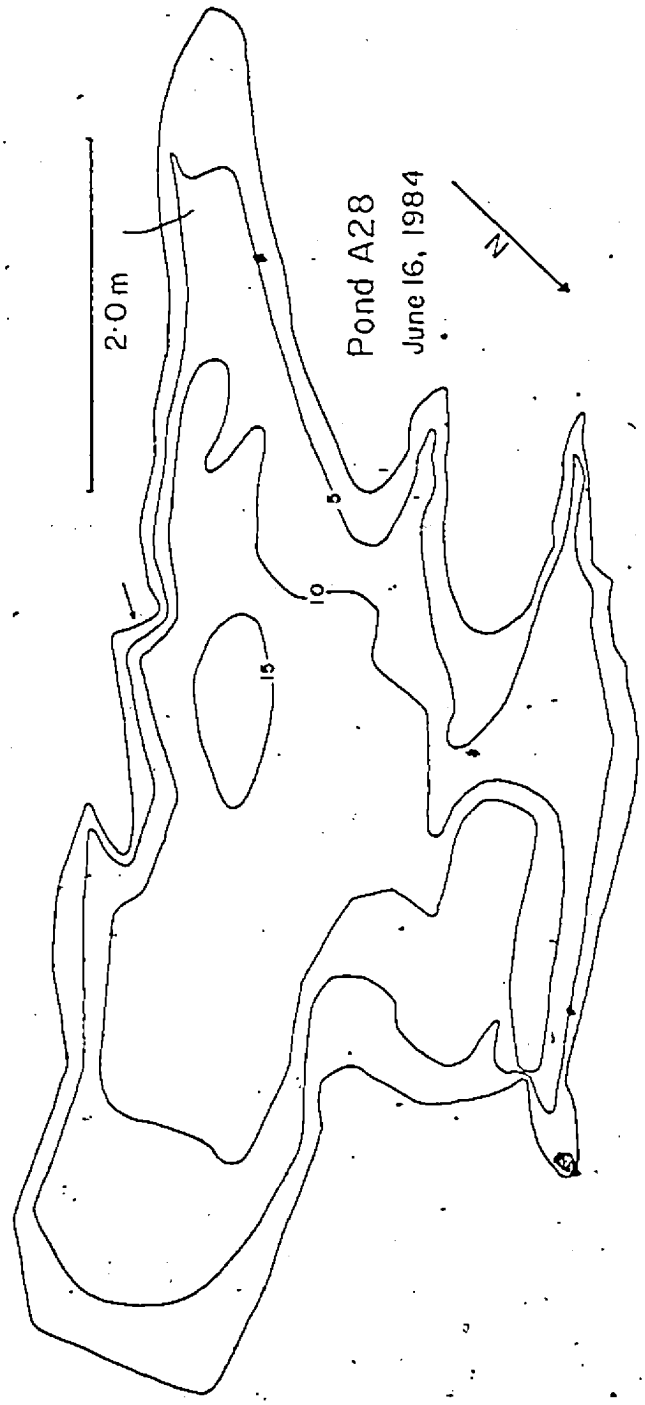
cladocerans, though more detailed studies from other species are necessary.

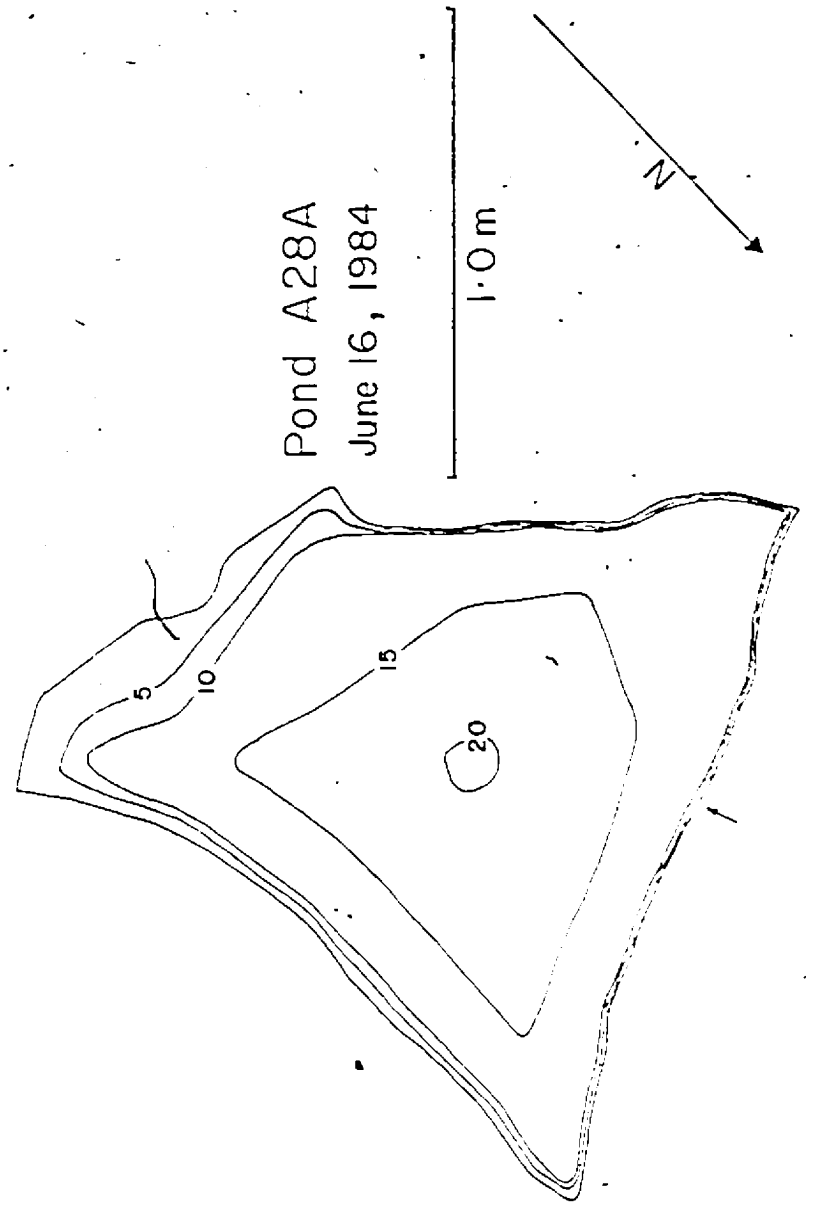
Environmental sex determination is probably a misleading description of the sex-determining mechanism in cladocerans in the sense that Charnov and Bull (1977) define the term. This does not imply, however, that the environment does not play an important role in inducing the appearance of the male sex, or the form of the population sex-allocation pattern. Obviously there is a need to determine the actual sex-determining mechanism for cladocerans. If this is accomplished it will be possible to examine the more interesting evolutionary question of how sex ratio is controlled in the Cladocera, a question that is still of interest for other cyclic parthenogens.

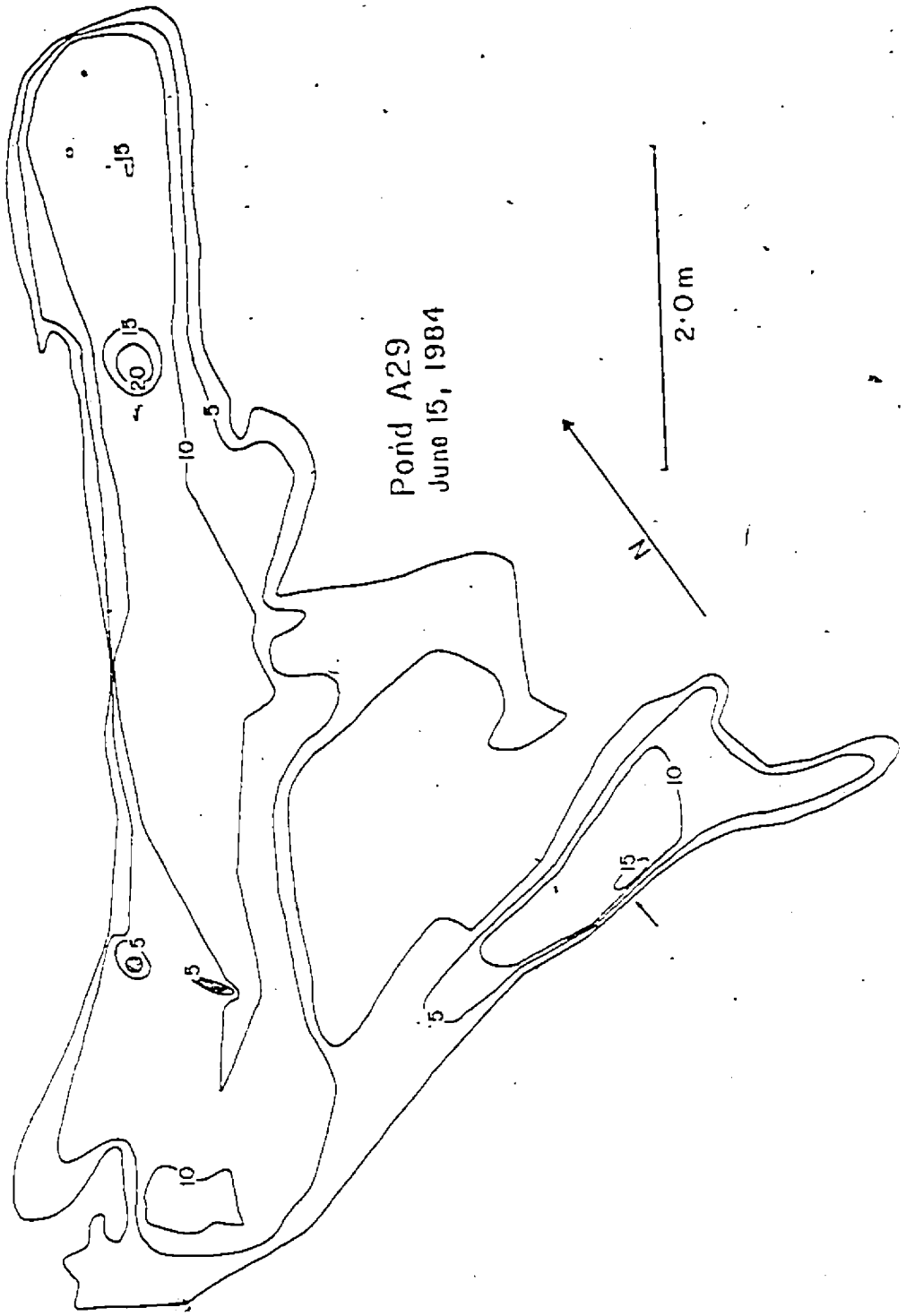
A final question is of considerable interest from the point of view of the evolution of sex ratio. There is a need to examine the degree of individual, or clonal, variation in SSR. This information will bear on the models of sex-ratio evolution, and the debate over selection for and against a high sex-ratio variance (Kolman 1960, Verner 1965, Burley 1982). As yet our knowledge of individual lifetime SSR's in cladocerans is limited to the data of this study. These data have not provided us with a clear picture of individual sex-allocation patterns.

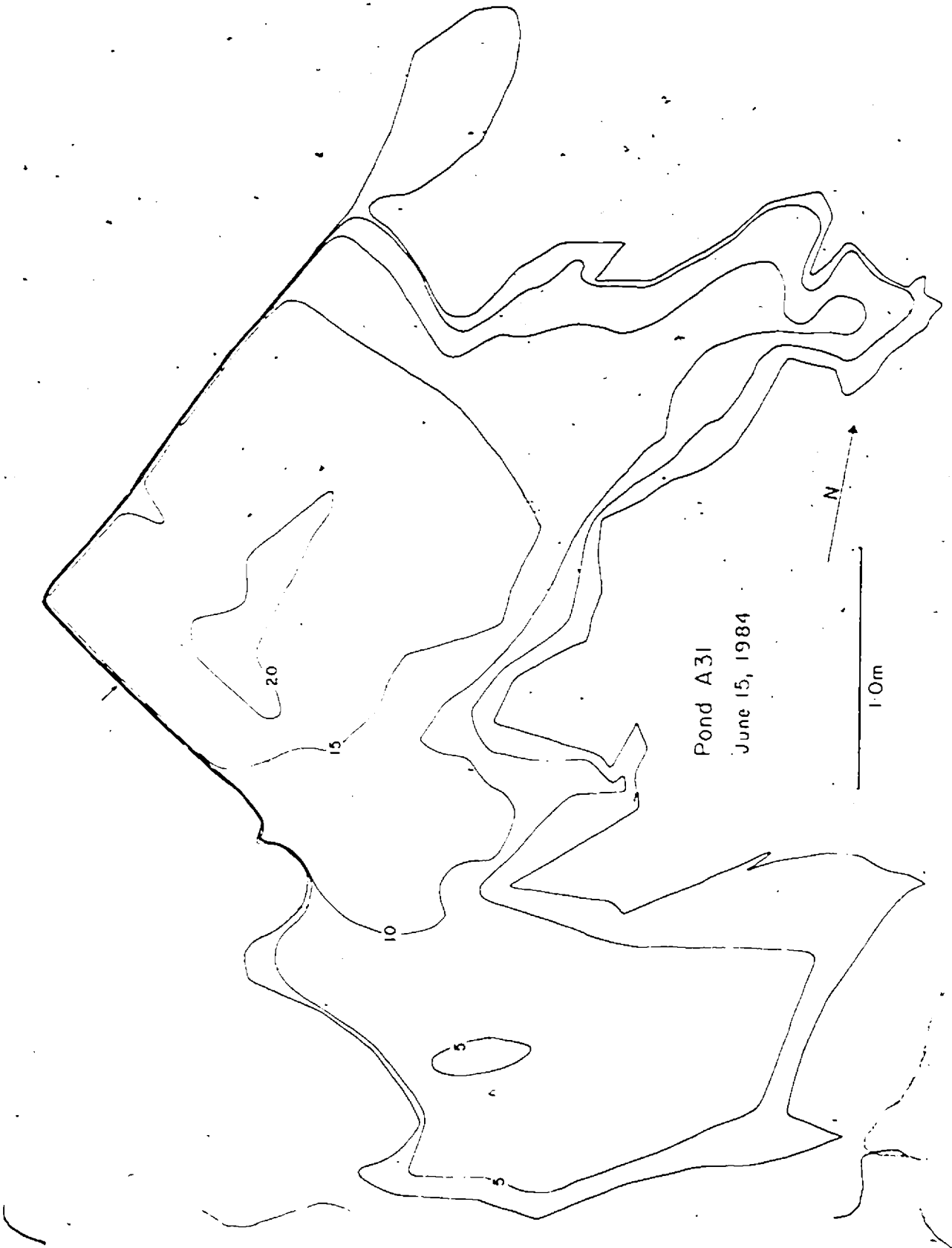
APPENDIX I

Survey maps of the four ponds (A28, A28A, A29, A31 (including A31A)) studied in detail in 1984. The pond boundary is drawn as it was found on the date of the map. The small arrow on each map points to the location of a black standard depth marker painted on the rock surface next to the pond, measurements are made from the lower edge of this marker to the water surface. On the map date these markers were exactly 10 cm (ponds A28, A29, A31), or 15 cm (pond A28A) above the water line. Direction arrows indicate magnetic north. True north was about 4 degrees to the east of magnetic north in 1984. Depth isoclines are given in centimetres. Stippled areas are emergent stones within the pond.









APPENDIX II

This appendix contains a summary of the raw data from 21 ponds on the numbers of individuals of 8 different reproductive types in pond samples. The reproductive types are ephippial females (EF), parthenogenetic females (PF), non-reproductive females (NRF), imminently parthenogenetic females (IPF), imminently ephippial females (IEF), males (M), juvenile males (JM), and juvenile females (JF). Their identification was made according to the criteria presented in the Methods. Three ratios have been calculated; the juvenile sex ratio (R1), or male juveniles over total juveniles, the mating sex ratio (R2), or adult males over imminently ephippial females plus males, and adult males over adult males plus ephippial plus imminently ephippial (R3). These proportion estimates are provided with their 95% confidence intervals. Dates of sampling are indicated for August (A), and September (S). The ponds were located on Bluff A (A), and the Churchill Bluff (CH).

APPENDIX II

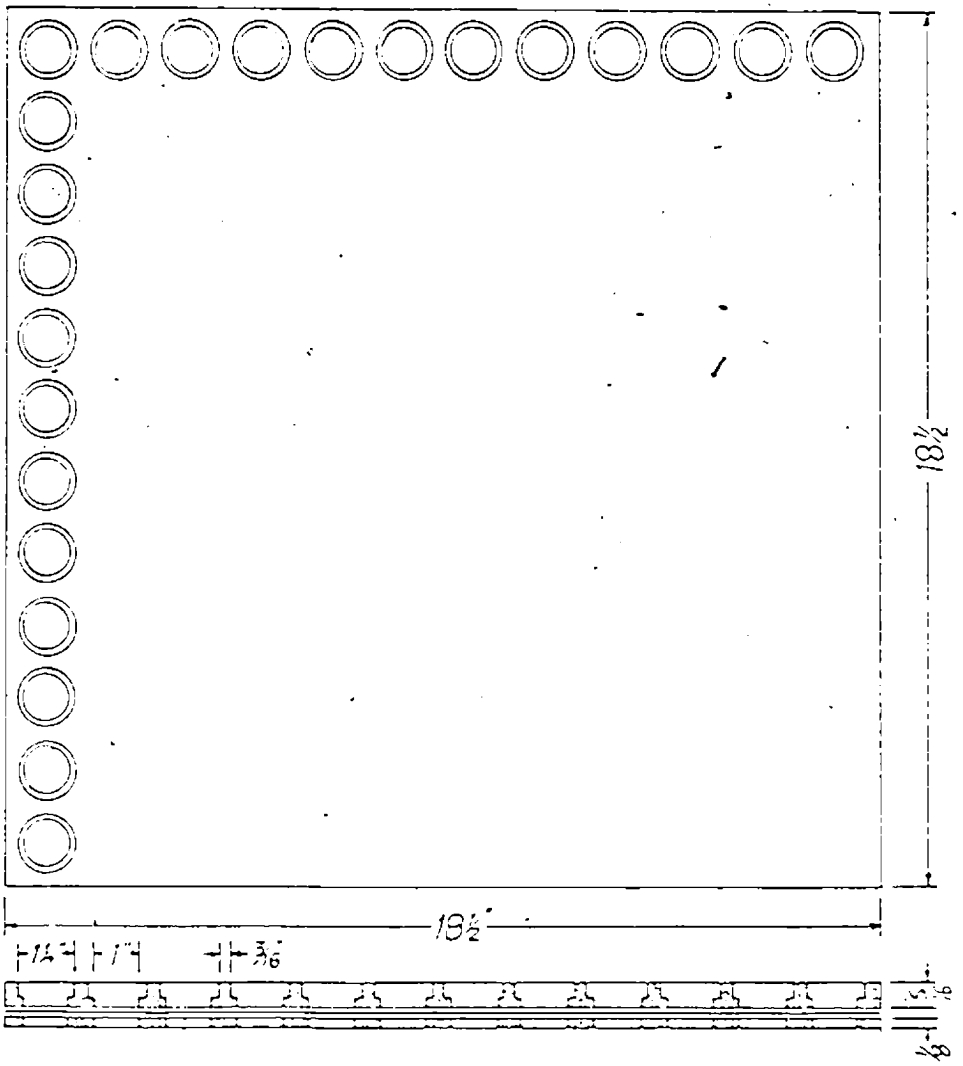
Pond	Day	Tot	EP	PP	NRP	IPP	IEP	M	JM	JP	U	R1	L	U	R2	L	U	R3	L
A31	A5	1129	11	229	55	---	14	32	127	661	.187	.161	.135	.822	.696	.542	.688	.561	.426
	A7	2514	45	519	123	---	31	41	191	1564	.125	.109	.095	.688	.569	.446	.442	.350	.265
	A22	305	0	23	17	36	5	20	70	134	.410	.343	.275	.932	.800	.593	.932	.800	.593
	S8	244	28	14	9	39	5	83	31	35	.598	.470	.345	.982	.943	.875	.798	.716	.631
	S8	292	22	26	5	44	5	69	31	90	.348	.256	.184	.978	.932	.850	.807	.719	.619
A29	A5	411	9	84	13	---	43	12	45	205	.233	.180	.135	.350	.218	.118	.306	.188	.099
	A7	1543	39	203	33	---	76	44	249	899	.246	.217	.196	.463	.367	.284	.356	.277	.212
	A20	872	24	2	27	26	80	49	198	466	.337	.298	.264	.469	.380	.297	.401	.320	.247
	A22	163	4	2	5	16	8	10	48	70	.503	.407	.321	.785	.556	.307	.678	.455	.244
	A27	393	42	51	13	69	19	143	23	33	.551	.411	.279	.926	.883	.840	.763	.701	.631
A28	A5	710	87	31	21	---	48	38	191	294	.436	.394	.345	.552	.442	.333	.290	.220	.160
	A8	1156	214	42	38	---	122	119	210	411	.378	.338	.303	.559	.494	.431	.303	.262	.219
	A13	1972	182	11	8	27	171	477	370	726	.369	.338	.311	.769	.736	.699	.604	.575	.536
	A13	1103	145	5	7	36	95	249	176	390	.350	.312	.271	.779	.724	.676	.565	.509	.475
A28A	A5	500	58	39	47	---	49	48	106	153	.474	.409	.349	.604	.495	.396	.389	.310	.238
	A8	1541	40	34	44	---	60	195	484	684	.441	.414	.379	.821	.765	.713	.713	.661	.603
	A13	615	49	29	0	8	22	144	178	187	.544	.488	.436	.917	.867	.810	.734	.670	.601
	A16	2492	260	66	5	22	173	553	532	881	.405	.377	.355	.791	.762	.728	.691	.661	.629
	A21	2017	187	9	11	20	136	441	457	756	.408	.377	.350	.796	.764	.724	.616	.577	.544
	A21	1322	100	9	7	16	62	188	414	526	.472	.440	.408	.802	.752	.692	.695	.637	.586
	A23	2794	139	7	39	28	138	454	832	1157	.442	.418	.398	.803	.767	.734	.655	.621	.584
	A28	247	8	3	21	1	7	40	56	111	.410	.375	.264	.938	.851	.717	.841	.727	.593
	A28	582	38	0	86	14	18	80	113	233	.382	.327	.280	.889	.816	.731	.674	.588	.503
	S10	588	44	1	19	186	15	107	106	110	.559	.491	.420	.932	.877	.808	.723	.645	.572
A30	A1	426	1	52	37	---	5	18	57	256	.227	.182	.137	.925	.783	.563	.902	.750	.533
	A25	446	48	35	29	96	14	115	39	80	.422	.328	.247	.938	.891	.823	.719	.650	.575
A23	A1	812	43	162	78	---	105	67	90	275	.299	.247	.205	.468	.390	.316	.378	.312	.248
A10	A1	113	2	26	11	---	3	12	24	35	.545	.407	.285	.957	.800	.519	.697	.706	.440
	A31	401	64	4	41	---	52	232	10	0	----	----	----	.864	.817	.770	.720	.667	.618
A36	A1	512	0	49	100	---	0	10	82	271	.278	.232	.186	1.00	1.00	.690	1.00	1.00	.690
	S5	270	20	1	53	95	5	87	2	7	.600	.222	.028	.982	.946	.875	.853	.777	.691
	S5	224	24	2	48	72	5	66	0	7	.410	0.00	0.00	.764	.930	.841	.781	.695	.587
A34	A24	452	72	11	8	71	61	97	40	90	.394	.308	.235	.606	.606	.530	.406	.418	.356
	A24	455	63	6	8	61	47	152	31	87	.348	.263	.184	.812	.764	.702	.641	.580	.488
CHA	A2	1385	73	117	85	---	61	130	247	662	.300	.272	.241	.746	.681	.609	.553	.492	.427
CHB	A2	1170	107	36	15	---	65	259	317	377	.498	.457	.423	.842	.799	.754	.646	.601	.552

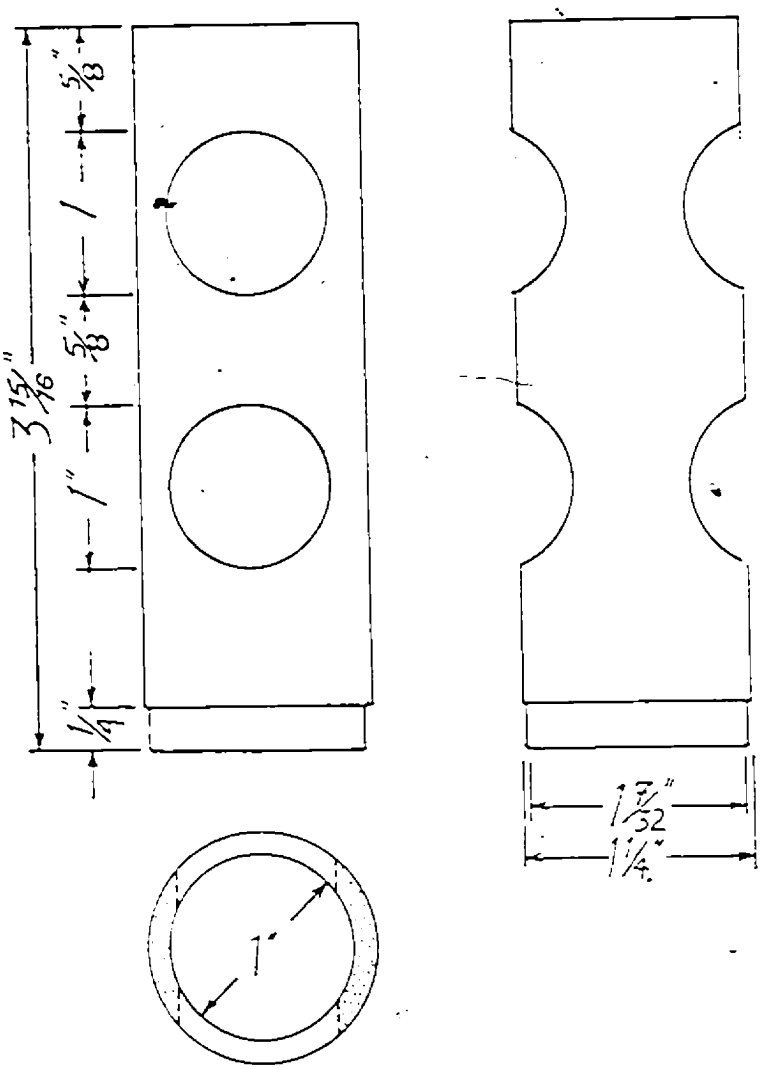
APPENDIX II (cont.)

Pond	Day	Tot	EP	PP	NRP	IPP	IEP	M	JH	JP	U	R1	L	U	R2	L	U	R3	L
CHC1	A9	1134	123	18	34	---	118	166	200	475	.336	.296	.265	.642	.585	.516	.460	.408	.361
CHC2	A2	817	84	16	48	---	39	163	242	225	.566	.518	.473	.862	.807	.749	.629	.570	.510
CHD	A2	1718	35	14	133	---	28	71	585	842	.435	.410	.385	.805	.717	.622	.610	.530	.441
CHE	A2	494	10	108	7	---	0	100	161	108	.661	.599	.537	1.00	1.00	.964	.956	.909	.839
CHF	A2	494	1	0	91	---	0	42	161	509	.274	.240	.208	1.00	1.00	.912	.994	.977	.882
CHG	A9	720	40	129	0	---	9	25	242	275	.514	.468	.426	.871	.735	.556	.459	.338	.235
CHH	A9	998	39	8	20	---	44	119	575	193	.780	.749	.718	.797	.730	.654	.659	.589	.519
CHX	A9	310	5	6	20	---	17	103	53	48	.626	.525	.423	.916	.858	.785	.883	.824	.742
T100	A1	1650	0	43	45	---	4	25	390	1143	.276	.254	.233	.961	.862	.684	.961	.862	.684
T102	A9	570	21	181	239	---	34	20	12	53	.300	.185	.099	.511	.370	.244	.385	.267	.174

APPENDIX III

A diagram of the enclosure utilized in the temporal cycle sex ratio control experiment. The base (first page) is constructed of two lucite sheets sandwiching a sheet of 64-μm Nitex. The two lucite sheets are bonded permanently with a cyanoacrylate glue. The base is perforated by an array (12 X 12) of holes which are spanned by the Nitex sheet. There are 144 "cells" (second page) which fit snugly into the base at their milled ends. Each cell has four side ports located on opposite sides in pairs. These ports were covered on the outside with 64-μm Nitex bonded to the cell's surface with transparent silicone cement. The enclosure is suspended in the pond with the base downwards and the cells projecting above the surface of the water to prevent escape of the detainees.





APPENDIX IV

	Brood Number					Total
	1	2	3	4	5	
Male Broods	30	25	18	15	8	96
Female Broods	122	63	31	17	5	238
Total	152	88	49	32	13	334
Proportion Male	0.20	0.28	0.37	0.47	0.62	

Proportions of male broods among the first to fifth broods produced by females during the endogenous cycle experiment performed in 1985. The temporal sequence of broods bears no relation to a standard time in the mother's life cycle, the standards were the time of beginning and end of the experiment. Sample size is of the number of first, second, etc. broods produced in the experiment. Brood series including ephippia were counted into the totals before the ephippial brood but not after. For these data $\chi^2 = 19.7$, $df = 4$, $p < .001$.

APPENDIX V

	Brood Number				
	1	2	3	4	5
Average Brood Size	24.4	16.6	11.3	11.3	5.4
95% Confidence Int. U	26.8	17.5	12.7	12.9	7.3
L	22.0	15.7	9.9	9.7	3.5
Standard Deviation	15.33	4.33	5.07	4.68	3.72
Number of Offspring	3782	1507	600	418	91
Number of Broods	155	91	53	37	17

Average brood sizes for the first to fifth broods of females from the endogenous cycle experiment performed in 1985. Brood series including an ephippial brood were truncated after the ephippial brood.

LITERATURE CITED

Allen, E. 1928. A note on the chromosomes of Moina macrocopa. Science 67:18.

Allen, E. and A.M. Banta. 1929. Growth and maturation of the parthenogenetic and sexual eggs of Moina macrocopa. J. Morphol. and Physiol. 48:123-151.

Bacci, G., G. Cognetti, and A.M. Vaccari. 1961. Endomeiosis and sex determination in Daphnia pulex. Experientia 17:505-506.

Banta, A.M. 1931. Further observations on the control of sex with Cladocera. Proc. 2nd. Int. Congr. for Sex Research (London, 1930). pp. 211-220.

Banta, A.M. 1937. Population density as related to sex and to evolution in the Cladocera. Am. Nat. 71:34-49.

Banta, A.M., and L.A. Brown. 1929. Control of sex in the Cladocera. III Localization of the critical period for control of sex. Proc. Natl. Acad. Sci. 15:71-81.

Banta, A.M. and C.A. Stuart. 1932. The critical period for control of sex in Moina. Proc. Soc. Exper. Biol. Med. 29:1253-1255.

Banta, A.M., T.R. Wood, L.A. Brown, and L. Ingle. 1939. Studies on the physiology, genetics, and evolution of some Cladocera. Carnegie Institution of Washington, Washington D.C., Dept. of Genetics, Paper No. 39.

Barker, D.M., and P.D.N. Hebert. 1986 Secondary sex ratio of the cyclic parthenogen Daphnia magna (Crustacea, Cladocera) in the Canadian arctic. Can. J. Zool., in press.

Bell, G. 1982. The masterpiece of nature; the evolution and genetics of sexuality. University of California Press, Berkeley.

Bodmer, W.F., and A.W.F. Edwards. 1960. Natural selection and the sex ratio. Ann Hum. Genet., Lond. 24:29-244.

Borror, D.J., D.M. DeLong, and C.A. Triplehorn. 1976. An introduction to the study of insects. 4th ed. Holt, Rinehart, and Winston, New York.

Bull, J.J. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, London.

Burley, N. 1982. Facultative sex-ratio manipulation. Am. Nat. 120:82-107.

Charniaux-Cotton, H. 1965. Hormonal control of sex differentiation in invertebrates. In R.L. DeHaan and H. Ursprung Eds. Organogenesis. Holt, Reinhart, and Winston, New York.

Charnov, E.L., and J.J. Bull. 1977. When is sex environmentally determined? Nature 266:828-830.

Crew, F.A.E. 1965. Sex-Determination. Methuen and Co., London.

Croxton, F.E. 1959. Elementary statistics with applications in medicine and the biological sciences. Dover, New York.

Ferrari, D.C., and P.D.N. Hebert. 1982. The induction of sexual reproduction in Daphnia magna: genetic differences between arctic and temperate populations. Can. J. Zool. 60:2143-2148.

Fisher, R.A. 1930. The genetical theory of natural selection. Oxford University Press, Oxford.

Flanders, S.E. 1946. Control of sex and sex-limited polymorphism in the Hymenoptera. Quart. Rev. Biol. 21:135-143.

Frey, D.G. 1982. Contrasting strategies of gamogenesis in northern and southern populations of Cladocera. Ecology 63:223-241.

Gilbert, J.J., and C.E. Williamson. 1983. Sexual dimorphism in zooplankton (Copepoda, Cladocera, and Rotifera). Ann. Rev. Ecol. Syst. 14:1-33.

Good, A.G. 1981. The ecology and biogeography of tundra zooplankton communities in the Churchill, Manitoba area. MSc. Thesis, University of Windsor.

Hamilton, W.D. 1967. Extraordinary sex ratios. Science 156:477-488.

Hebert, P.D.N. 1978. The population biology of Daphnia (Crustacea, Daphniidae). Biol. Rev. 53:387-426.

Hebert, P.D.N., and R.D. Ward. 1972. Inheritance during parthenogenesis in Daphnia magna. Genetics 71:639-642.

Kolman, W.A. 1960. The mechanism of natural selection for the sex ratio. Am. Nat. 94:373-377.

Korpelainen, H. 1985. Ecological and evolutionary genetics of Daphnia (Crustacea, Cladocera). PhD. Diss., University of Helsinki,

Leary, D. 1967. Induction of males and ephippial eggs in Daphnia pulex. MSc. Thesis, University of Maryland.

Lees, A.D. 1959. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid Megoura viciae Buckton - I The influence of these factors on apterous virginoparae and their progeny. J. Insect. Physiol. 3:92-117.

Lees, A.D. 1960. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid Megoura viciae Buckton - II The operation of the "interval timer" in young clones. J. Insect Physiol. 4:154-175.

Lumer, H. 1937. Growth and maturation in the parthenogenetic and sexual eggs of Daphnia magna Staus. Cytologia 8:1-14.

MacGillivray, M.E., and G.B. Anderson. 1964. The effect of photoperiod and temperature on the production of gamic and agamic forms in Macrosiphum euphorbiae (Thomas). Can. J. Zool. 42:491-510.

Mainland, D., L. Herrera, and M.I. Sutcliffe. 1956. Statistical tables for use with binomial samples -- contingency tests, confidence limits, and sample size estimates. Dept. of Medical Statistics, New York University College of Medicine, New York.

Mayo, Z.B., and K.J. Starks. 1972. Sexuality of the greenbug, Schizaphis graminum, in Oklahoma. Ann. Ent. Soc. Amer. 65:671-675.

Morgan, T.H. 1909a. A biological and cytological study of sex determination in phylloxerans and aphids. J. Exper. Zool. 7:239-353.

Morgan, T.H. 1909b. Sex determination and parthenogenesis in phylloxerans and aphids. Science N.S. 29:234-237.

Morgan, T.H. 1915. The predetermination of sex in phylloxerans and aphids. J. Exper. Zool. 19:285-321.

Ojima, Y. 1958. A cytological study on the development and maturation of the parthenogenetic and sexual eggs of Daphnia pulex (Crustacea,Cladocera). Kwansei Gakuin Univ. Ann. Studies 6:123-171.

Reinhard, E.G. 1949. Experiments on the determination and differentiation of sex in the bopyrid Stegophyrxus hyptius Thompson. Biol Bull. 96:17-31.

Ruvinski, A.O., S.F. Cellarius, and Yu. G. Cellarius. 1978. The possible role of genome activity changes in the sex determination of Daphnia pulex. Theor. Appl. Genet. 52:269-271.

Schwartz, S.S., and P.D.N. Hebert. 1986a. Breeding system of Daphniopsis ephemeralis: adaptations to a transient environment. Hydrobiologia, in press.

Schwartz, S.S., and P.D.N. Hebert. 1986b. Daphniopsis ephemeralis sp. n. (Cladocera,Daphniidae): a new genus for North America. Can. J. Zool., in press.

Scourfield, D.J. 1943. The post-embryonal development of the male of Daphnia magna. J. Queckett Microscop. Soc., Ser. 4, 1:276-283.

Shaw, R.F., and J.D. Mohler. 1953. The selective significance of the sex ratio. Am. Nat. 87:337-

Shull, A.F. 1925. Sex and the parthenogenetic-bisexual cycle. Am. Nat. 59:138-154.

Shull, A.F. 1929. Determination of types of individuals in aphids, rotifers, and Cladocera. Biol. Rev. 4:218-248.

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. 2nd. ed. W.H. Freeman and Co., San Francisco.

Stimpfl, K.J. 1971. The effects of four environmental variables on the induction of gamogenesis in Daphnia pulex Leydig. PhD. Diss., Indiana University.

Trivers, R.L., and D.E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

Verner, J. 1965. Selection for sex ratio. Am. Nat. 99:419-421.

White, M.J.D. 1973. Animal cytology and evolution, 3rd. ed.. Cambridge University Press, Cambridge.

Young, J.P.W. 1983. The population structure of cyclic parthenogens. In G.S. Oxford, and D. Rollinson (Eds.), Protein polymorphism: adaptive and taxonomic significance. Academic Press, London. pp. 361-378.

VITA AUCTORIS.

David Montgomery Barker

Born: June 6, 1959
Etobicoke, Ontario

Elementary School:	Maple Grove Public School Oakville, Ontario 1972
Secondary School:	Oakville Trafalgar High School Oakville, Ontario 1977
Bachelor of Science:	Biology Queen's University Kingston, Ontario 1981
Master of Science	Biology University of Windsor Windsor, Ontario 1986